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# CHEMICAL COMPOSITION OF THE SHELL OF EXTERNAL AND INTERNAL PARASITIC EGGS (HYMENOPTERA) AND THEIR HOST EGGS AND PENETRATION OF CHEMICALS THROUGH IT

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## ABSTRACT

The work has been carried out on the chemical composition of shell and embryonic membranes of eggs of *Microbracon gelechiae* Ashm., *Corcyra cephalonica* Staint., *Chilo zonellus* S., *Pyrilla perpusilla* Walk., *Trichogramma evanescens minutum* Riley and *Tetrastichus pyrrillae* Craw., and penetration of chemicals through them. The eggs of the latter two insects are endoparasitic in eggs of *C. cephalonica* as well as *C. zonellus* and *P. perpusilla* respectively. The eggs of *M. gelechiae* are ectoparasitic on larvae of *Gnorimoschema operculella* Zell. and *C. cephalonica*.

The endoparasitic eggs of *T. evanescens minutum* and *T. pyrrillae* have thin non-resistant proteinaceous chorion and epembryonic membrane. They are devoid of the internal lipid layer. They are not resistant to the penetration of hydrophilic or lipophilic substances. The poison that gets into the host-eggs is enough to kill them. In eggs of rest of the insects there is a remarkable parallelism in their fundamental structure. The shell bears respiratory pores, running through its substance. It consists of external proteinaceous cement containing loose admixture of unsaturated oily substance, proteinaceous chorion made of non-resistant exochorion and resistant endochorion, and a lipid layer of unsaturated oily material internal to the chorion in freshly laid eggs—and of a mixture of unsaturated and saturated fatty substances in older eggs. The chorion and the lipid layer are fully completed in the ovary. The latter is formed by ovum and the former by follicular cells. In some of the eggs of *M. gelechiae* the lipid layer is completed after oviposition. The micropyle is closed by lipid from this layer immediately after fertilisation and before oviposition. The cement is deposited after fertilisation when the eggs are ready for oviposition. The proteinaceous vitelline and fertilisation membranes are devoid of any oil, but the serosa and later the epembryonic membrane contain a small quantity of unsaturated-lipoid in older eggs, and it is reabsorbed by the developing larva before hatching.

The cement and the chorion do not obstruct the penetration of hydrophilic or lipophilic poisons into the eggs; they often retain some poison to cause the death of those larvae which eat their way through while hatching. The main obstruction to the entry of water-soluble substances is given by the lipid layer; but it is supplemented to some extent when some fatty material appears in the epembryonic membrane in mid-development, and when some of the unsaturated-fat of the lipid layer is converted to saturated state. The lipophilic substances penetrate quite easily through all layers of the eggs; but some resistance encountered by them and a fumigant hydrogen cyanide during mid-development may be due to the fully developed state of epembryonic membrane, which is even resistant to the action of some corrosive chemicals at this time.

## INTRODUCTION

The investigations have been carried out on eggs of *Microbracon gelechiae* Ashm., *Trichogramma evanescens minutum* Riley (Hymenoptera), *Chilo zonellus* S., *Corcyra cephalonica* Staint. (Lepidoptera), *Tetrastichus pyrrillae* Craw. (Hymenoptera) and *Pyrilla perpusilla* Walk. (Homoptera). The first one is an external parasite of larvae of *Gnorimoschema operculella* Zell., a pest of potatoes (Narayanan, 1948a), and the second one is an internal parasite of eggs of maize stem borer, *C. zonellus* (Narayanan, 1948b). The larvae and the eggs of *C. cephalonica* serve as a laboratory host for the first and second insects respectively (Narayanan and

Mookherjee, 1955). *P. perpusilla* is a serious pest of sugarcane and *T. pyrrillae* is the endo-parasite of its eggs (Narayanan and Kundan Lal, 1953). The work described herein is meant to be a study of differences in the chemical composition, modifications of the egg-envelopes, if any, during development because of their different environmental conditions, and to indicate how toxic materials and simple chemicals penetrate from the outside to the inner living contents. It is not intended to devise methods of control or to obtain ovicidal materials, but this study may provide a 'model' for parasitic (Hymenopterous) and host (Lepidopterous and Homopterous) eggs in the same way that previous work (Beament, 1946a,b : 1948, 1949, 1951 and Beament and Rattan Lal, 1957) has indicated the broad principles governing the mechanism of penetration through the eggs of *Rhodnius prolixus* Ståhl. and *Pieris brassicae* Linn. It also explains how the host egg provides protection to the delicate egg of the parasite developing within the former. It may be possible that these recent fundamental studies and those undertaken in future may be of some value in selecting the ovicides effective against the eggs of pests, but not of parasites.

#### MATERIAL AND METHOD

The eggs of *M. gelechiae* are obtained by the usual established practice of confining male and female adults in 3×2 inches glass dishes containing soaked raisins and covered on one side with muslin, the host larvae of *C. cephalonica* being kept on top of the muslin covered over with a glass plate. The insects of all the species used in this work are reared at about 30°C and 75 per cent relative humidity in an incubator. The females of *M. gelechiae* deposit eggs through the muslin-partition scattered on the surface of the larvae. The eggs can be removed gently from the host larvae with a fine camel-hair brush, as they lie loose and not gummed to the surface. In the absence of host if the eggs are crowded the newly hatched larvae suck juice from the unhatched eggs or even from the sluggish larvae. Generally the unmated females do not lay eggs; but sometimes the unfertilised eggs are deposited by them which do not hatch at all and consequently there is no ovoviviparous development in the insect. The eggs laid by mated females are removed from the host and kept in the incubator until needed. The eggs hatch within 24 hours at 30°C of 100 per cent R.H., indicating a uniformity of development; one can therefore presume that the eggs are fertilised immediately before laying.

The eggs of *C. cephalonica* are obtained by the usual methods of keeping gravid females in tin containers having glass top and wire-gauze bottom. These containers are placed in trays. The females oviposit through the wire-gauze and the eggs dropped in the tray below are collected. The eggs of *C. zonellus* and *P. perpusilla* are obtained by confining the gravid females in large cages containing maize and sugarcane plants respectively and kept in the field. Both the insects laid eggs in batches. The eggs of *C. zonellus* remain firmly gummed to the leaf surface, and for chemical tests they are kept soaked in water for some time and then removed from the leaf with camel-hair brush. Acetone or any other solvent cannot safely dislodge them from the leaf. Because of highly adhesive cement most of the eggs get damaged while removing them from the leaf; hence for tests where living eggs are needed they are not dislodged from the leaf pieces. The batches of eggs of *P. perpusilla* are covered over with waxy secretion and detached hairs from the female (Narayanan, 1953). These eggs, though firmly attached to the leaf surface, can be easily removed from it with camel-hair brush soaked in acetone without damaging them. This method loosens their cement and the waxy covering. The eggs of these insects are kept in an incubator until needed. The duration of egg-stage of *C. cephalonica*, *C. zonellus* and *P. perpusilla* at 30°C and 100 per cent R.H. is about 3, 4 and 9 days respectively.

The eggs of *T. evanescens minutum* (Narayanan and Mookherjee, 1955) and those of *T. pyrrillae* (Narayanan and Kundan Lal, 1953) are obtained by confining the eggs of the respective host along with the male and female adults in 2×4 inches glass-stoppered jars kept in an incubator. The parasites oviposit inside the host eggs, which are dissected to remove the parasitic eggs. The removal of parasitic eggs from the host eggs at different intervals after parasitisation indicates that the duration of egg stage of the parasites is about 24 hours at 30°C.

The technique employed is after Beament and Rattan Lal (1957) with slight modification where necessary. The experimentation with eggs is carried out at 30°C and 100 per cent R.H. The external morphology of the eggs is studied in whole mounts as well as portions of the shell mounted in glycerine, whereas the penetration experiments are conducted with the staining method. In some of the latter experiments radioactive phosphorus,  $P^{32}$ , is also used. The eggs without treatment with lipid-solvent or after treatment for some time with petroleum-ether or chloroform are immersed for 5 and 60 minutes in 0.5 per cent aqueous solution of phosphoric acid ( $H_3PO_4$ ) containing 250 $\mu$ c. of radioactive  $P^{32}$ . Later these eggs are washed with water and assayed for radioactivity. The conversion of some of the unsaturated material of lipid layer of the eggs to saturated state during egg-development is studied as follows:—Fifty eggs or ova are cut into longitudinal halves, removing the inner contents along with epembryonic or vitelline membrane and thoroughly washing with distilled water. These are quickly treated with 0.1 ml. of chloroform in cavity slide. The treated shells are removed and the chloroform after evaporation leaves a deposit of fatty material on the slide. This fatty deposit is treated with 0.01 ml. of 0.1 per cent aqueous iodine solution, and the process of decolourisation of the iodine solution is noted after one hour and three hours. Each experiment is repeated six times. The air spaces in cement or respiratory pores in chorion of the eggs are studied by injection with cobalt naphthenate, the method recommended by Wigglesworth (1950) and Wigglesworth and Beament (1950). The 'transition temperature' of the eggs, i.e. the temperature at which the lipid layer becomes markedly more permeable to water, has been determined with the technique adopted by Beament (1951) and Judge (1953). To determine the active or passive mechanism of absorption and retention of moisture within the eggs, one thousand ova, living eggs and those killed with hydrogen sulphide are separately desiccated for four hours in four replications at 30°C and 0 per cent relative humidity. The difference in weight of the eggs before and after desiccation gives the amount of water lost. The desiccated eggs are then brought to 75 per cent or 100 per cent R.H. to find out whether they can regain the lost water.

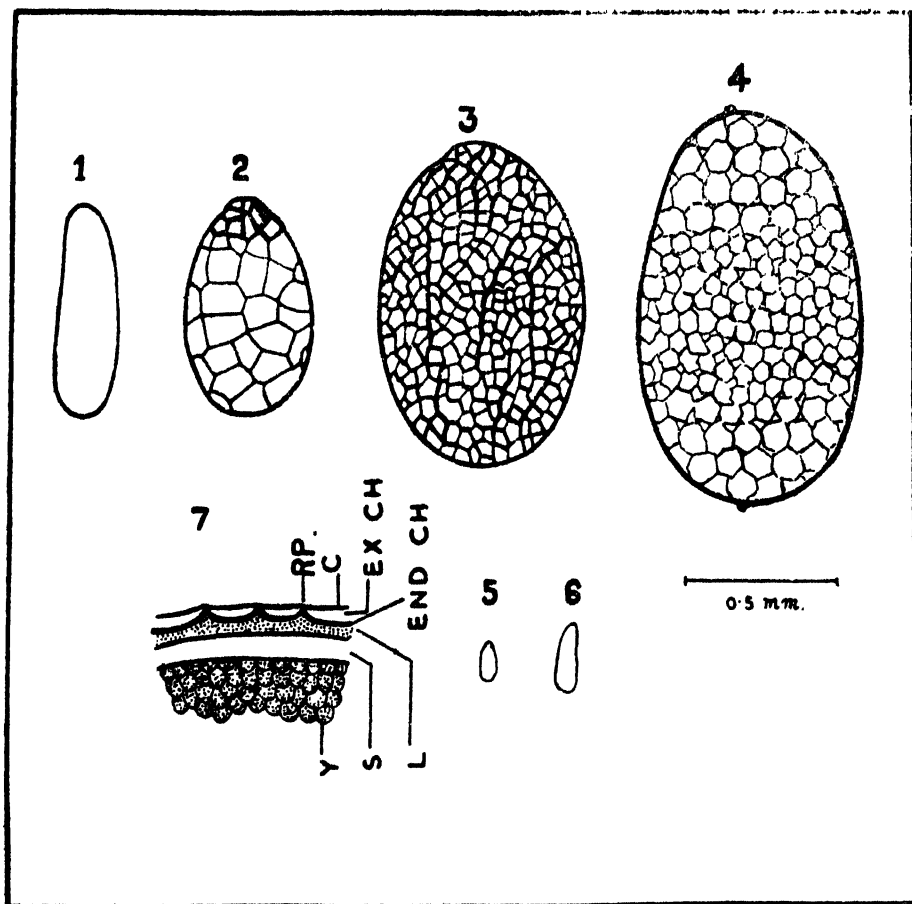
The procedure for ovicidal experiments is after Beament (1948) and the tests are performed with water-soluble salts and salts both water- and lipid-soluble. The eggs are gently forced into the ovicidal solution with a camel-hair brush because of the presence of air in respiratory pores. They are kept dipped for five minutes and then the excess of the solution from their surface is removed with pieces of filter paper. They are then kept in an incubator. Thirty eggs per test with six replications and along a control-set constitute a single experiment.

## PART I: MORPHOLOGY OF EGGS, PHYSICAL AND CHEMICAL PROPERTIES OF THEIR SHELL, THEIR WATER AND OXYGEN RELATIONS

### (a) *Morphological notes on the eggs*

A freshly laid egg of *M. gelechiæ* (Fig. 1) is dirty white, has 3.5 microns thick shell and measures 0.607 by 0.187 mm.; that of *C. cephalonica* (Fig. 2) is opaque, has 5.8 microns thick shell and measures 0.583 by 0.373 mm.; that of *T. evanescens minutum* (Fig. 5) as well as of *T. pyrrillae* (Fig. 6) is transparent, has 1.2 micron

thick shell and measures 0.117 by 0.047 mm. and 0.233 by 0.07 mm. respectively; that of *C. zonellus* (Fig. 3) is light yellowish, has 4.7 microns thick shell and measures 1.003 by 0.607 mm.; and that of *P. perpusilla* (Fig. 4) is whitish, giving reddish to greenish hues, has 6.3 microns thick shell and measures 1.143 by 0.56 mm. All these eggs have a micropyle at the anterior end. They get proportionately darkened with age due to the changes in the developing embryo; the latter is somewhat discernible through the semitransparent shell. The egg-shell of *M. gelechiae*, *T. evanescens minutum* and *T. pyrillae* is quite smooth externally, whereas that of *C. cephalonica*, *C. zonellus* and *P. perpusilla* bears lines depicting the impressions of the boundaries of follicular cells of the ovary. This pattern



TEXT-FIG 1.

Fig. 1, *Microbracon gelechiae* Ashm. egg.

Fig. 2, *Corcyra cephalonica* Staint egg.

Fig. 3, *Chilo zonellus* Zell. egg.

Fig. 4, *Pyrilla perpusilla* Walk. egg.

Fig. 5, *Trichogramma evanescens minutum* Riley egg.

Fig. 6, *Tetrastichus pyrillae* Craw. egg.

Fig. 7, Diagrammatic sketch of transverse section through portion of an egg, depicting fundamental layers. C: Cement, CH: Chorion, END.CH: Endochorion, EX.CH: Exochorion, L: Lipoid layer, R.P: Respiratory pore, S: Serosa, Y: Yolk.

which is composed of non-resistant exochorion, is very mild and honey-combed type on the egg of *P. perpusilla*, but it is quite prominent and zig-zag on that of

*C. zonellus* and *C. cephalonica*. Further in the latter insect these lines are comparatively thicker at the anterior end in a little area round the micropyle, and this area when seen from above seems to give the appearance of a sieve-cap lying on top of the egg. Moreover the egg of *P. perpusilla* is slightly flattened dorso-ventrally. There is a small knob at its each end, the anterior one being larger than the posterior. The former and the latter measure 0.036 by 0.05 mm. and 0.02 by 0.033 mm. respectively. The micropylar canal traverses through the anterior knob and opens on the tip. The posterior knob is a solid structure.

#### (b) Construction of the shell membranes

The chorion of the eggs of *M. gelechiae*, *C. cephalonica*, *C. zonellus* and *P. perpusilla* consists of two layers: an external proteinaceous coating, the exochorion and an internal layer of tanned protein, the endochorion (Fig. 7). Internally the lipid layer of oily material is situated between the chorion and vitelline membrane, and the latter is afterwards transformed into serosa (Fig. 7), and then into epembryonic membrane, containing a little quantity of lipid, and is ultimately reabsorbed before hatching. On the outer surface of the egg is spread by the female with a cement (Fig. 7), a proteinaceous secretion of the accessory gland, which in the eggs of *M. gelechiae* and *C. cephalonica* dries up quickly; whereas in *C. zonellus* and *P. perpusilla* it fixes the shell firmly to the leaf surface. Moreover in the latter it appears to be firmly held by the stalks of the anterior and posterior knobs.

The chorion of the egg of *T. evanescens minutum* and *T. pyrillae* consists of only a single layer of non-resistant proteinaceous material. There is a complete absence of internal lipid layer. The vitelline membrane as usual is transformed into serosa and then epembryonic membrane, which is devoid of the traces of lipid and is reabsorbed before hatching. It is difficult to say whether the external cement layer (the usual lubricating layer of Hymenopterous eggs) is present or absent on these eggs; as the fully formed ova which are always devoid of the cement could not be obtained by dissecting such small insects and studied along side with the eggs. It is also not possible to dissect out the lubricating glands of these small insects even if they are present.

#### (1) The cement layer

The cement on outer surface of egg-shell of *M. gelechiae* is not present in sufficient quantity to facilitate chemical and physical tests. Most of the work has therefore been done on the paste-like contents of the cement gland (called the lubricating gland in this insect) of the female. On exposing a thin layer of this material to air, it gets transformed to a substance behaving in every way like the layer on the shell, which indicates that no chemical addition is made at the time of oviposition. It is a cement of non-adhesive type and loses only a little quantity of water on exposure to air, but gives off the entire water on desiccation and simultaneously its protein gets denatured. When the dried cement is brought back to saturated relative humidity it regains the lost water, but it does not become paste-like again. It is hygroscopic to some extent and consequently maintains a higher concentration of moisture outside the shell, thereby preventing the latter from too much drying. The contents of the gland cannot be diluted with water after they are exposed to air. It could be assumed from this that some denaturation of cement-protein has taken place by its drying in air. This process is apparently speeded up by the chemical denaturants like ethylalcohol or phosphomolybdic acid, but not by pure oxygen alone. In the gland the paste-like fluid having a pH of 7.5 consists of a colourless matrix containing a large number of irregular granules of dirty white colour and lipid globules. When exposed

to air it sets into a layer; the minute granules, the fluid and the lipid globules get mixed up and unite in such a way as to assume the appearance of a membrane. The process of denaturation affects the fluid part greatly which binds the other constituents. The lipid globules in the cement layer do not acquire a uniform disposition, with the result that some spaces are left which are devoid of the lipid material. The cement does not prevent the solutions of water- and oil-soluble stains from reaching the chorion surface, provided they could wet the outer surface of the egg. It may also be mentioned that the presence of lipid material in the cement also helps in lubricating the oviduct and the ovipositor of the female, thus facilitating an easy passage of eggs through them. That is why the cement in such insects is referred to as lubricating material and the gland secreting it as the lubricating gland.

The staining reactions with fuchsin, orange-G, safranin as well as tests like xanthoproteic and ninhydrin have shown that the matrix and granules of the cement are acidophillic protein. The presence of a little reducing substance in these is indicated by argentaffin test. The cement, not only reduces osmic acid, but also gets stained with sudan fat stains. Corrosive substances such as strong nitric acid or sodium hypochlorite break down the cement, releasing oily droplets and thus indicating it to be formed of lipoprotein. But solvents like chloroform and ether can remove the lipid component of the cement and the extracted material gives a strong reducing action. It is therefore obvious that it is an unsaturated lipid, not chemically bound to the protein component. It does not contain any water-soluble reducing material attached to a protein. This cement is attacked by strong formic acid and sulphuric acid, a characteristic not common to most insect shell components. The treatment with lipid solvents does not produce any marked visual change in the cement, but it becomes a little brittle on drying and shows much stronger colouring with aqueous stains. The removal of lipid from it affects somewhat its water retentive power. The lipid therefore is of considerable importance in maintaining a balance in the moisture content of the cement and the surrounding air.

The other gland associated with the sexual organs in a female *M. gelechiae* is the 'poison gland'. Its proteinaceous secretion is markedly acidic with pH 4.0 and contains a small quantity of unsaturated lipid. The globular cells around the poison duct also secrete an unsaturated lipid which is employed in lubricating the inner passage of the ovipositor prior to stinging the host. The secretions of the globular cells, the main poison gland and the lubricating gland soften the chorion only to a little extent.

A similar series of tests have been performed on the contents of the cement glands (accessory glands) of the females of *C. cephalonica*, *C. zonellus* and *P. perpusilla*. The chemical constituents of the cement from these insects are the same as those of the cement of *M. gelechiae*. The cement from *C. cephalonica* contains less lipid than that of *M. gelechiae*, but it resembles the latter in physical properties. The cement from *C. zonellus* and *P. perpusilla* has air spaces in its substance like a sponge and is highly adhesive. It also contains less lipid than that of *M. gelechiae*. The cement from all these insects does not contain any water-soluble reducing substance. Moreover the waxy covering on a batch of eggs of *P. perpusilla* helps them to resist desiccation and penetration with solutions of aqueous materials.

## (2) The chorion

The chorion of a fully formed ovum and of a newly laid egg of *M. gelechiae* is soft and plastic. It remains so if it is kept in contact with moisture, but when exposed to low humidity or heated to 40°C there appears some irreversible hardening in it; and this takes place in nature by normal exposure to atmosphere. The

pliability of chorion is related to its water-content and is not altered by extraction with chloroform. Chorion is composed of two layers, a comparatively thick exochorion and a thin endochorion (Fig. 7), which cannot be separated mechanically or chemically. Both these layers are made of minute particles embedded in matrix. Chorion is perforated by small pores (Fig. 7) scattered all over it. The blocking of these pores by the application of heavy petroleum oil, such as petroleum jelly, liquid paraffin, etc. on the surface of chorion actually kills the developing embryo by suffocation, thus indicating that these are respiratory pores. A study of the different stages of ovum in ovariole tube indicates that the chorion is deposited by the follicular cells.

Both the parts of chorion are basically formed of protein as is evident from staining reactions with orange-G, safranin, fuchsin, picric acid, iodine as well as from the xanthoproteic and ninhydrin tests. They do not colour with sudan fat stains or osmic acid. The fatty droplets are not liberated when these are acted upon by strong acids, etc.; and there is no intensification of staining reactions after these are extracted with chloroform. Thus, no free or chemically bound lipid exists in them. They give argentaffin and *p*-benzoquinone reactions, but these are more pronounced in endochorion than those in exochorion, and more so in the granular parts of these layers than in the matrix. Thus they appear to be partially quinone tanned protein. Moreover, the exochorion dissolves more readily in strong acid, and is therefore regarded as less cross-linked. Strong nitric acid breaks down the chorion in cold, releasing the small particles which eventually are dissolved within a few hours. When slowly acting material, such as dilute mineral acids, strong ammonia, urea, strong formic acid, pepsin and trichloroacetic acid are used they produce no effect on either layer. Trypsin on the other hand digests the exochorion only. This therefore confirms the suggestion that the endochorion is of tanned substance, whereas the exochorion is free from such bonds. A solution of lithium iodide or sodium thioglycolate does not materially affect the resistance of chorion to solution and therefore it appears unlikely that sulphur bonds play any substantial part in their construction. Both the layers of chorion do not prevent quite big molecules of the stains from penetrating into the interior of the egg, since they colour with water-soluble stains even when the entire egg is dipped in the solution, and since the developing embryo gets stained even though both the layers remain uncoloured when the entire lot is immersed in oil-soluble stains. The exochorion does not undergo any change after the egg is laid, but the tanned endochorion of ova inside the female is readily soluble in strong ammonia and 10 per cent potassium hydroxide. It seems that much of the tanning of the endochorion goes on subsequent to fertilisation, but is completed within a few hours of embryonic development. The embryo has nothing to do with it, as the tanning process of endochorion is completed even in an ovum after removal from the ovary and incubated under similar conditions. Thus the excess tanning material probably already present in the endochorion continues its action even after the eggs are laid.

A similar study of the exo- and endo-chorion of the eggs of *C. cephalonica*, *C. zonellus* and *P. perpusilla* indicates that the chemical nature resembles that of *M. gelechiae*. Like the latter the respiratory pores traversing the chorion are scattered in the eggs of *P. perpusilla*, whereas these are concentrated to some extent near the anterior end of the eggs of *C. cephalonica* and *C. zonellus*. In the eggs of *T. evanescens minutum* and *T. pyrrillae*, the chorion is very thin and resembles to some extent physically and chemically the exochorion of *M. gelechiae*, except that it does not seem to have any phenolic tanning material and the respiratory pores are absent. It is just possible that these differences might be due to the fact that these eggs are never exposed to atmospheric conditions and they remain embedded in the tissue of host eggs after they are laid.



(3) *The lipoid layer*

The lipoid layer in an egg of *M. gelechiae* lies between the endochorion and the outer embryonic layer (Fig. 7). It is present on a small scale in ova but definitely in the oviposited eggs. This substance is a freely mobile material. After extraction with chloroform, the oily residue blackens deeply with osmic acid, and decolourises iodine solution. It is obviously an unsaturated material. It may be mentioned here that the lipoid from ova or the newly laid eggs decolourises the iodine solution more quickly than that obtained from the eggs in an advanced stage of development (Table I). This indicates that the ova and freshly laid eggs contain more unsaturated lipoid-material than the eggs of advanced stages; and during the course of development the embryo produces some effect on it (may be catalytic oxidation) and some of its oily material is altered to saturated oil. But this process of oxidation needs assistance of the developing embryo and does not

TABLE I

*Decolourisation of iodine solution by the fatty material from the lipoid-layer of eggs of the following insects*

|   | <i>M. gelechiae</i> |   |   |   |   |   | <i>C. cephalonica</i> |   |   |   |   |   | <i>C. zonellus</i> |   |   |   |   |   | <i>P. perpusilla</i> |   |    |   |   |   |
|---|---------------------|---|---|---|---|---|-----------------------|---|---|---|---|---|--------------------|---|---|---|---|---|----------------------|---|----|---|---|---|
|   | a                   | b | c | d | e | f | a                     | b | c | d | e | f | a                  | b | c | d | e | f | a                    | b | *c | d | e | f |
| Unsaturated lipoid decolourising iodine solution after (Hours) :— | 1                   | 1 | 1 | 3 | 3 | 3 | 1                     | 1 | 1 | 3 | 3 | 3 | 1                  | 1 | 1 | 3 | 3 | 3 | 1                    | 1 | 1  | 3 | 3 | 3 |

*Inference*:—The delay in decolourisation of iodine solution indicates that some of the unsaturated fatty material of the lipoid layer has been altered to saturated state.

*Note* :—Fatty material from the lipoid layer of :

- a. Ova fully formed.
- b. Eggs freshly laid.
- c. Ova fully formed but these have been earlier incubated at 30°C and 100 per cent R.H. for the period equivalent to half incubation period of a normal egg.
- \*c. As in 'c' excepting that ova incubated for the period equivalent to about quarter incubation period of a normal egg.
- d. Eggs in mid-development.
- e. Eggs about to hatch.
- f. Egg-shell after hatching.

appear to take place of its own accord, as there is no difference in the decolourisation of iodine solution with the lipoid taken from the ova incubated for half the incubation period of the normal eggs, from that of the lipoid taken from the fresh ova. Since it is extremely difficult to obtain this lipoid in large quantity to undertake detailed chemical analysis, the investigation on this aspect will be taken up some time later. This unsaturated lipoid giving water-proofing to eggs of *M. gelechiae* is unique so far as our present knowledge of apparently water-proofing lipoid substances of the cuticle is concerned. The lipoid layer gives chief obstruction to the penetration of only water-soluble materials into the egg and not of the oil-soluble substances. This point is further confirmed beyond doubt by experimenting with phosphoric acid containing the radioactive phosphorus, P<sup>32</sup>. The eggs, which are not given prior treatment with ether or chloroform and are immersed only for 5 minutes in the solution of phosphoric acid, do not show any radioactivity, but those eggs which are immersed for 60 minutes show radioactivity in the shell as well as in the inner contents. This is probably due to the fact that phosphoric acid which is only slightly soluble in oil-solvent like ether fails to

penetrate into the eggs with 5 minutes' immersion, whereas it enters the eggs that remain dipped for an hour. On the other hand the contents of the eggs whose lipid layer has been treated earlier with petroleum-ether or chloroform do show radioactivity whether the eggs are immersed in the phosphoric acid solution for 5 minutes or for an hour. This indicates that ether or chloroform treatment of the eggs by dislodging the lipid layer facilitates the entry of highly water-soluble phosphoric acid. Thus the lipid layer provides a barrier to the penetration into the eggs of water-soluble materials which are only slightly soluble in oil-solvent when the immersion is of short duration, whereas in longer immersions the little solubility of such materials in lipid-solvent helps appreciably the process of penetration.

The formation of the lipid layer commences in the follicular tube. Ova in different stages of growth are stained with sudan fat stains. It is noticed that the lipid is only concentrated in the substance of the growing ovum, whereas it is present in traces in follicular cells. The lipid appears to be deposited by ovum on the surface of vitelline membrane, whereas the chorion is formed on it simultaneously by the follicular cells. The chorion at no time is found to contain any oily material. Sometimes the formation of the lipid layer is not completed in an ovum and it is completed within an hour or so after the oviposition. The aqueous stain in solution penetrates quite easily into the freshly laid eggs in which the lipid layer is incomplete, whereas in those eggs in which it is already complete the penetration of such materials does not take place. The fertilisation of ovum has nothing to do with the completion of the lipid layer, as it is even completed in ova separated from the ovary and incubated under similar conditions. The transition temperature varies between 50°C and 60°C and it is not particularly sharp. It is variable in different eggs. However, there is some increase in it as the egg advances in age. This rise in transition temperature may be due to the conversion of some of the unsaturated lipid to saturated state, and also may be due to some loss of the more volatile component from the layer. But both these suggestions need further investigation.

In eggs of *C. cephalonica*, *C. zonellus* and *P. perpusilla* the lipid layer internal to the chorion is also formed of freely mobile unsaturated oily material, which is deposited by the ovum before the eggs are laid. As in the eggs of *M. gelechiac*, some of its unsaturated oil gets converted into saturated oily substance in the advanced stages of eggs by the developing embryo (evident from iodine decolourisation experiments, Table I). This phenomenon also exists in the eggs of *P. brassicae* re-examined now. The transition temperature of these eggs is not sharp and falls between 60°C to 70°C. It comes nearer to the upper limit as the eggs grow older, as is the case in the eggs of *M. gelechiae*. The experiments with the dyes have shown that in these eggs also the lipid layer is a potential barrier to the penetration of water-soluble materials. The tests with phosphoric acid containing radioactive phosphorus,  $P^{32}$ , have indicated that the water-soluble substances having a little oil-solubility can penetrate the eggs with longer immersions. In eggs of *T. evanescens minutum* and *T. pyrrillae* the lipid layer is absent, and therefore solutions of water-soluble materials can easily penetrate the shell to reach the embryo. It is just possible that since these eggs remain immersed in the fluid of host eggs, there is no need for any water-proofing in them. The solutions of oil-soluble substances also easily enter these eggs.

### (c) Embryonic covering

It is worthwhile to probe into the nature of extra-embryonic membrane interior to the lipid layer so as to find out whether any changes in it are running parallel with changes in the oviducal resistance of the eggs. The ovum of *M. gelechiae*, *C. cephalonica*, *C. zonellus* and *P. perpusilla* is surrounded by vitelline membrane, which changes into fertilisation membrane in a fertilised egg. It i

proteinaceous and is devoid of lipid in any form either free or chemically bound. It is readily permeable to water-soluble and oil-soluble materials in solution. Some time after oviposition it is progressively replaced by serosa (Fig. 7) and then by epembryonic membrane, when it becomes a little more resistant to the action of corrosive substances such as strong hydrochloric acid, 10 per cent potassium hydroxide, or 5-7 per cent sodium hypochlorite in cold, than the vitelline or fertilisation membranes. Even at this time it is quickly soluble in strong nitric acid; and is permeable to some extent to water-soluble and oil-soluble substances. Its chemical nature is somewhat similar to that of exochorion, except that it is less resistant to the corrosive substances than the latter, contains a little unsaturated lipid, but does not contain any tanning material. Thus at this stage it forms to some extent a secondary water-proofing mechanism. A re-examination of this membrane of *P. brassicae* now has indicated that it also contains a little quantity of unsaturated lipid which escaped detection earlier. Moreover, when the larva is about to hatch the resistance of this membrane diminishes, probably due to the softening caused by some chemical action from the living material; it, however, does not revert completely to the properties of the original vitelline or fertilisation membranes.

To explain the changes in the resistance of epembryonic membrane with age, the effect of pre-treatment of eggs of these insects with trichloroacetic acid, and sodium thioglycolate on the action of trypsin has been studied. In the freshly laid eggs and those near hatching the membrane is soluble in cold trichloroacetic acid, whereas it is insoluble in it in the intervening stages of development, thus showing the preparation for hatching carried out on the membrane by the embryo. Similarly, trypsin at pH 8.5-8.8 and 37°C disintegrates the early and late membrane, but not in the intervening period. When the membrane in the more resistant stage is treated first with sodium thioglycolate at 30°C and pH 12, it is not dissolved, but following this treatment it is readily broken down by both trypsin and trichloroacetic acid. Similarly concentrated lithium iodide solution makes the membrane of the resistant stage slowly soluble in trichloroacetic acid, and not-disintegrable in trypsin. In view of this the actual chemical linkage in this membrane may be a form of thioquinone. The rate of changes in its resistance is inversely proportional to the incubation period of the eggs. But owing to the small size of these eggs and the minuteness of the material under study it has not been possible to undertake detailed chemical analysis which could settle this point.

In the unfertilised eggs the vitelline membrane is applied closely and quite securely to the upper lip of the micropyle. The lipid layer is not therefore complete round the ovum, and sperm can enter from micropyle directly into the surface of the vitelline membrane. The lipid layer in these eggs is completed round the micropylar end immediately after fertilisation and before oviposition, consequently the deposited eggs are quite resistant to the penetration of water-soluble materials and to water-loss. The completion of the lipid layer at micropylar end may be due to a slight shrinkage of the egg-cell leading to the breakage of the junction between micropyle and vitelline membrane and flowing of the lipid across to seal the micropyle. In the few eggs of *M. gelechia* having incomplete lipid layer for some time after oviposition, the resistance to penetration of water-soluble substances is somewhat feeble at that time.

The epembryonic membrane of the eggs of *T. evanescens minutum* and *T. pyrillae*, like their shell behaves like ordinary denatured protein. It is devoid of lipid and is reabsorbed before hatching.

#### (d) Water relations of the eggs

The eggs and newly hatched larvae of *M. gelechia* contain about 90 per cent and 88 per cent water by weight respectively. The amount of moisture in cement

layer could not be quantitatively determined, as it is applied just like a varnish on egg's surface. The cement being hygroscopic provides moist surroundings to the chorion. The unfertilised eggs (mature ova) lose water rapidly on desiccation (Table II) and their rate of water-loss during four hours' desiccation is 5 per cent by weight per hour, whereas that of the fertilised eggs only about 1 to 1.5 per cent. There is no significant difference between the rates of loss of normal eggs and ova under the similar conditions as compared with those killed with hydrogen sulphide prior to desiccation. It appears, therefore, that death does not produce

TABLE II

*Percentage loss of water in dry air at 30°C from living eggs and ova, or those killed by fumigation with hydrogen sulphide for half an hour*

| Age of eggs<br>(Hours) | Alive         |                    | Dead          |                    |
|------------------------|---------------|--------------------|---------------|--------------------|
|                        | In 4<br>hours | % rate<br>per hour | In 4<br>hours | % rate<br>per hour |
| 1-2                    | 6.2           | 1.55               | 6.6           | 1.65               |
| 4                      | 4.1           | 1.02               | 3.9           | 0.97               |
| 8                      | 4.5           | 1.12               | 4.2           | 1.05               |
| 12                     | 4.2           | 1.05               | 4.1           | 1.02               |
| 24                     | 5.9           | 1.47               | 6.2           | 1.55               |
| Mature ova from ovary  | 20.1          | 5.02               | 20.3          | 5.07               |

any 'break down' in the water-proofing mechanism of the eggs. But the process of fertilisation and after oviposition the subsequent stages up to mid-development are accompanied by some irreversible improvement in water-proofing. The latter may be correlated to the completion of the lipid layer in those eggs in which it has not been completed within the ovary on the one hand, and to the conversion of some of the unsaturated lipid of this layer to saturated state as well as to the deposition of some oily material in the epembryonic membrane with development on the other. Prior to hatching the water-proof nature reverts back to some extent to the state which is seen in freshly laid eggs, and it is correlated to the disintegration and reabsorption of the membrane by the embryo at this time. Moreover if the living or dead eggs desiccated in different stages of development are kept at 75 per cent or 100 per cent relative humidity they do not regain the lost water, indicating that there does not exist any active or passive mechanism for water absorption.

In *T. evanescens minutum* and *T. pyrrillae* the eggs after removal from the host eggs do not survive desiccation even at 75 per cent R.H. and this is due to the absence of water-proofing lipid layer.

The eggs and newly hatched larvae of *C. cephalonica*, *C. zonellus* and *P. perpallia* contain about 86 per cent and 82 per cent water by weight respectively. The cement accounts for nearly 2 per cent of the total weight of a freshly laid egg of the first and nearly 9 per cent of that of a newly laid egg of the last two insects. The living or dead eggs of these insects in different stages of development, if desiccated and later brought to 75 to 100 per cent R.H. absorb a quantity of water which can only be accounted for by the absorption with the cement, indicating that even in these eggs there is no other active or passive mechanism for the absorption of moisture. Moreover in these eggs also there is some improvement in the water-proofed nature in the mid-development age, which disappears prior to hatching.

(c) *Oxygen relations of the eggs*

Dipping for about six hours in water or even up to 12 hours is not fatal to the eggs, which indicates that this does not interfere in their respiration. If they are kept immersed for a long period they die because of restriction in oxygen supply. They could complete development under water if the latter is kept aerated with air or even oxygen or if 5 volumes of hydrogen peroxide are mixed with 95 of water ; but most of the larvae die soon after hatching. The aqueous solutions of a wetting agent (Teepol) is lethal as compared with similar volumes of distilled water if the eggs are dipped for a long period. It cannot be imagined that oxygen diffusion through either of them differs, but the solution of wetting agent slowly displaces air from the respiratory pores which might be acting as a physical-gill in the immersed eggs. Even the lethal effect of the obstruction of respiratory pores by short immersion in heavy petroleum-oil can be eliminated by removing it from the surface of the eggs with petroleum-ether. Thus there is a need for the respiratory pores to remain open for the normal development of the eggs. The eggs of *C. cephalonica*, *C. zonellus* and *P. perpusilla* could complete development in an atmosphere containing more than 50 per cent oxygen, whereas those of *M. gelechiae* fail to do so at such a high concentration of oxygen.

## PART II : OVICIDAL EXPERIMENTS

The experiments given herein are only intended to give an idea about the types of poisons which can penetrate through egg-envelopes and destroy the developing embryo, and to indicate whether the parasitic-egg developing within the host-egg could be saved from such poisons or not.

(a) *Water-soluble salts of heavy metals*

The chlorides and acetates of cobalt, copper, nickel and manganese used in concentrations of 1 per cent in water do not produce any harmful effect on eggs of *M. gelechiae*, *P. perpusilla*, *C. cephalonica* and *C. zonellus*. The mortality of larvae during hatching or some time afterwards is quite common in the latter two insects, which is due to the action of poisonous materials they consume when they eat a portion of the shell for hatching. There are hardly any post-hatching casualties in the former two insects, as they do not consume the poison from the shell since they only pierce their way through for hatching. These substances do not become any more efficient either when the eggs are immersed for 5 minutes following evacuation of air from the respiratory pores and forcing the liquid into them, or when they are used in combination with a wetting agent (1 per cent Teepol). It appears that these purely water-soluble chemicals with no oil-solubility, which are potentially very toxic, are not able to cross the barrier of the lipid layer and reach the embryo. These water-soluble poisons have also failed to kill the eggs of *T. evanescens minutum* and *T. pyrillae* developing in their respective host-eggs. The parasitic eggs are permeable to water-soluble substances due to the absence of lipid layer in them; but the protection they get while they are within the host-eggs is due to the latter possessing a non-permeable inner lipid layer.

The freshly laid eggs of *M. gelechiae*, *P. perpusilla*, *C. cephalonica* and *C. zonellus* are a little more susceptible, especially when the wetting agent is mixed with the solution and the immersion period is raised to six hours (Table III). The wetting agent even alone when used for such a long duration does cause some harm to these eggs as well as to endo-parasitic eggs present within them. This is probably due to the fact that in longer immersions the small quantity of the wetting agent taken up by the chorion of the host-eggs remains in contact with their lipid layer for longer time and causes some disruption in it; but even in such conditions

the entire lipid layer is not disintegrated. Moreover solutions of glucose and sodium chloride (potentially harmless materials) of the same concentrations as well as of the concentrations chemically equivalent to those of the solutions of the chemicals used in the ovidical tests, do not have any detrimental effect on the eggs. This indicates that these concentrations of the chemicals in solution do not cause an adverse effect on the eggs through exosmosis during the dipping

TABLE III

*Percentage kill of freshly laid eggs following immersion for six hours in 1% aqueous solutions of the chemicals*

| Chemicals                | With<br>or<br>without<br>1%<br>Teepol | <i>M.</i><br><i>gele-</i><br><i>chia</i> | <i>P.</i><br><i>perpu-</i><br><i>silla</i> | <i>C.</i><br><i>cepha-</i><br><i>lonica</i> | <i>C.</i><br><i>zone-</i><br><i>llus</i> | <i>T. evanescens</i><br><i>minutum</i><br>eggs parasi-<br>tising eggs<br>of <i>C. cepha-</i><br><i>lonica</i> | <i>T. pyrrillae</i><br>eggs parasi-<br>tising eggs<br>of <i>P. perpu-</i><br><i>silla</i> |
|--------------------------|---------------------------------------|--|--|---|--|---|---|
| Cobalt<br>acetate        | a<br>b                                | 59<br>9                                  | 58<br>8                                    | 60<br>10                                    | 58<br>8                                  | 60<br>10  | 59<br>9   |
| Cobalt<br>chloride       | a<br>b                                | 59<br>9                                  | 58<br>8                                    | 58<br>8                                     | 56<br>6                                  | 59<br>9   | 60<br>10  |
| Cupric<br>acetate        | a<br>b                                | 90<br>20                                 | 86<br>18                                   | 82<br>16                                    | 84<br>17                                 | 86<br>18  | 82<br>16  |
| Cupric<br>Chloride       | a<br>b                                | 60<br>10                                 | 56<br>6                                    | 58<br>8                                     | 57<br>7                                  | 59<br>9   | 56<br>6   |
| Nickel<br>acetate        | a<br>b                                | 70<br>10                                 | 68<br>8                                    | 69<br>9                                     | 68<br>8                                  | 67<br>7   | 68<br>8   |
| Nickel<br>chloride       | a<br>b                                | 69<br>9                                  | 69<br>9                                    | 68<br>8                                     | 67<br>7                                  | 69<br>9   | 68<br>8   |
| Manganese<br>acetate     | a<br>b                                | 58<br>8                                  | 56<br>6                                    | 56<br>6                                     | 58<br>8                                  | 59<br>9   | 59<br>9   |
| Manganese<br>chloride    | a<br>b                                | 60<br>10                                 | 58<br>8                                    | 57<br>7                                     | 59<br>9                                  | 58<br>8   | 60<br>10  |
| Glucose<br>1%            | a<br>b                                | 42<br>9                                  | 37<br>6                                    | 40<br>10                                    | 37<br>7                                  | 38<br>8   | 40<br>10  |
| Glucose<br>1.2%          | a<br>b                                | 39<br>8                                  | 34<br>8                                    | 38<br>9                                     | 36<br>6                                  | 40<br>9   | 36<br>6   |
| Sodium<br>chloride 1%    | a<br>b                                | 37<br>10                                 | 39<br>6                                    | 36<br>6                                     | 36<br>8                                  | 40<br>8   | 39<br>8   |
| Sodium<br>chloride 0.36% | a<br>b                                | 38<br>9                                  | 38<br>7                                    | 40<br>7                                     | 38<br>6                                  | 39<br>10  | 36<br>10  |
| Teepol only 1%           |                                       | 40                                       | 35   | 39  | 38                                       | 38  | 36  |
| Water only               |                                       | 3  | 2  | 3   | 2  | 2   | 4   |
| No treatment             |                                       | 2  | 3  | 3   | 2  | 0   | 0   |

Note :—a. With Teepol.

b. Without Teepol.

period. Further copper acetate under similar conditions is more toxic to the eggs as well as to those of the internal parasites within them than copper chloride or

acetates and chlorides of the other elements; and this is probably due to the property of copper acetate being slightly soluble in lipid-solvent (like ether), whereas the other salts are insoluble.

(b) *Chemicals—having oil- and water-solubility*

A few experiments on eggs of these insects have been conducted to compare the toxicity of aqueous solutions of mercuric chloride, bromide and nitrate, lithium chloride, arsenic trichloride (water- and lipid-soluble compounds) and mercuric acetate (water-soluble compound). The results (Table IV) indicate that mercuric

TABLE IV

*Percentage kill of eggs following immersion for 5 minutes in 0.5% aqueous solutions of the chemicals*

| Chemicals                       | Stage of eggs | <i>M. gelechiæ</i> | <i>P. perpusilla</i> | <i>O. cephalonica</i> | <i>C. zonellus</i> | <i>T. evanescens</i><br>minutum<br>eggs parasitising eggs of <i>O. cephalonica</i> | <i>T. pyrrillæ</i><br>eggs parasitising eggs of <i>P. perpusilla</i> |
|---------------------------------|---------------|--------------------|----------------------|-----------------------|--------------------|--|--|
| Mercuric chloride               | a             | 100                | 100                  | 100                   | 100                | 100  | 100  |
|                                 | b             | 91                 | 89                   | 87                    | 90                 | 88   | 91   |
|                                 | c             | 97                 | 95                   | 93                    | 96                 | 94   | 97   |
| Mercuric bromide                | a             | 100                | 100                  | 100                   | 100                | 100  | 100  |
|                                 | b             | 87                 | 84                   | 83                    | 86                 | 96   | 95   |
|                                 | c             | 93                 | 90                   | 89                    | 92                 | 100  | 100  |
| Mercuric nitrate                | a             | 90                 | 88                   | 85                    | 87                 | 92   | 91   |
|                                 | b             | 61                 | 57                   | 59                    | 58                 | 60   | 56   |
|                                 | c             | 79                 | 77                   | 74                    | 76                 | 81   | 80   |
| Arsenic trichloride             | a             | 100                | 100                  | 100                   | 100                | 100  | 100  |
|                                 | b             | 85                 | 86                   | 83                    | 84                 | 87   | 85   |
|                                 | c             | 91                 | 92                   | 89                    | 90                 | 93   | 91   |
| Mercuric acetate                | a             | 10                 | 8                    | 7                     | 9                  | 10   | 11   |
|                                 | b             | 12                 | 10                   | 8                     | 11                 | 9  | 10   |
|                                 | c             | 10                 | 11                   | 10                    | 8                  | 9  | 11   |
| Arsenic trioxide, pH 6-7        | a             | 7                  | 9                    | 8                     | 6                  | 7  | 8  |
|                                 | b             | 8                  | 8                    | 7                     | 9                  | 7  | 9  |
|                                 | c             | 7                  | 7                    | 8                     | 9                  | 8  | 9  |
| Arsenic trioxide, pH 10 or more | a             | 100                | 100                  | 100                   | 100                | 100  | 100  |
|                                 | b             | 89                 | 90                   | 88                    | 88                 | 87   | 89   |
|                                 | c             | 95                 | 96                   | 94                    | 93                 | 94   | 95   |
| Lithium chloride                | a             | 6                  | 7                    | 7                     | 8                  | 6  | 7  |
|                                 | b             | 7                  | 8                    | 6                     | 8                  | 7  | 8  |
|                                 | c             | 6                  | 7                    | 7                     | 6                  | 8  | 9  |
| Water only                      | a             | 7                  | 8                    | 6                     | 9                  | 8  | 7  |
|                                 | b             | 9                  | 7                    | 9                     | 8                  | 7  | 8  |
|                                 | c             | 8                  | 7                    | 6                     | 9                  | 8  | 7  |
| No treatment                    | a             | 6                  | 7                    | 7                     | 8                  | 6  | 6  |
|                                 | b             | 7                  | 8                    | 8                     | 9                  | 7  | 7  |
|                                 | c             | 6                  | 5                    | 7                     | 8                  | 7  | 9  |

Note :—a. Eggs freshly laid.      b. Eggs in mid-development.      c. Eggs about to hatch.

chloride, bromide and arsenic trichloride are highly toxic to the eggs of all the insects as well as to the parasitic eggs found within the host-eggs, whereas mercuric nitrate is a little less toxic. The freshly laid eggs and those about to hatch are slightly more susceptible than the eggs in mid-development. The lithium chloride does not appear to be toxic to the eggs. Moreover, arsenic trioxide (a purely water-soluble salt) at pH 10 or more seems to kill the eggs, whereas it is non-toxic at 6 to 7 pH; this might be due to the fact that the higher pH probably produces some effect on the chorion and the lipid layer of the eggs and thereby facilitates the penetration of the chemical.

### (c) Fumigation of eggs

Hydrogen cyanide at atmospheric pressure penetrates more slowly in the eggs in mid-development than in freshly laid eggs or those about to hatch. Hydrogen sulphide is equally fatal at all stages of egg-development and has a quick action. All stages of the eggs are equally susceptible to mercury vapour, but in this case a little longer exposure is necessary.

## DISCUSSION

The study of structure and chemical composition of the shell consisting of cement, chorion and lipid, and embryonic membranes of eggs of *M. gelechiae*, *C. cephalonica*, *C. zonellus* and *P. perpusilla* as well as the information from the ovidical experiments indicate a similarity in fundamentals with the results obtained from eggs of *P. brassicae* by Beament and Rattan Lal (1957), but some interesting variations are also evident. The external waxy covering which is only present on the batches of eggs of *P. perpusilla* gives them an additional advantage in resisting desiccation and penetration with solutions of aqueous materials. The proteinaceous cement of the eggs of all these insects resembles that of a mite, *Metatetranychus ulmi* Koch. (Beament, 1951) and *P. brassicae* in having only a physical mixture of unsaturated lipid with its substance. It differs from that of *P. brassicae* in not containing any water-soluble reducing material linked to a protein. The cement of *M. gelechiae* like that of *Diataraxia oleracea* Linn. (Salkeld and Potter, 1953) contains more lipid than that found in the cement of the other insects. The cement is non-adhesive and devoid of air spaces in *M. gelechiae* and *C. cephalonica*, whereas in *C. zonellus* and *P. perpusilla* it is adhesive and spongy like that of *P. brassicae*. It is not possible to say whether the cement layer is present or absent from the eggs of *T. evanescens minutum* and *T. pyrillae* as it could not be studied. It may be pointed out here that the secretion of the globular cells of poison duct does not soften the chorion of eggs of *M. gelechiae* to the extent (i.e. 1/20th of its diameter for facilitating their passage through that much size of ovipositor canal) visualised by Narayanan and Subba Rao (1955). Even the secretions of the main poison gland and the lubricating gland (cement gland) do not produce this effect. To decide this point one has to search for explanation elsewhere, especially either the mechanism of the ovipositor or something else which has escaped notice so far.

The proteinaceous chorion of eggs of these insects and that of *P. brassicae* consisting of non-resistant exochorion and resistant endochorion is slightly different from that of *D. oleracea* in not having a layer corresponding to the thin exochorion of lipoprotein which covers the latter eggs. The respiratory pores in chorion of *C. cephalonica* and *C. zonellus* like the pores in that of *D. oleracea* and *P. brassicae* are somewhat concentrated at the anterior end of the eggs; whereas the pores are scattered all over the surface of the eggs of *M. gelechiae* and *P. perpusilla*. Like the



shell of eggs of *R. prolixus* (Beament, 1946a, 1948), ticks *Ixodes ricini* L. and *Ornithodoros moubuta* Murray (Lees and Beament, 1948), and *P. brassicae*, which is not water-proof, the chorion of all these insects is freely permeable to quite big molecules of hydrophilic and lipophilic stains and chemicals in solutions. The non-water-proof chorion of *T. evanescens minutum* and *T. pyrillae* differs from that of all the other insects in being very thin, consisting only of non-resistant protein, and is devoid of respiratory pores.

Like eggs of *P. brassicae* there is a lipid layer on the inner side of chorion of eggs of these insects, but it is absent from those of *T. evanescens minutum* and *T. pyrillae*. It has also been demonstrated in eggs of *Melinophus differentialis* Thom. by Slifer (1937, 1946), *R. prolixus* by Beament (1946b), *Locustana pardalina* Walk. by Matthee (1951), whereas it has escaped the notice of Roonwal (1954) and Sander (1956) in eggs of *Schistocerca gregaria* Forsk. and *P. perpallida* respectively. In *Psylla mali* Schm. (Beament, unpublished work), *P. brassicae* and the insects studied now, the lipid layer is formed of freely mobile fatty material and not a hard wax as has been earlier noticed in eggs of a mite *M. ulmi* (Beament, 1951), *R. prolixus* and *M. differentialis*. The present investigation has shown that some of its unsaturated-lipoid is converted into saturated-lipoid by the developing embryo. This phenomenon has been observed now even in the eggs of *P. brassicae* which earlier escaped detection. Among the insects having lipid layer the transition temperature of eggs of *M. gelechiae* lies between 50°C and 60°C, whereas that of the other insects like *P. brassicae* varies from 60°C to 70°C.

The resistance of embryonic membranes of the insects under study to the action of corrosive chemicals is lower in the early and late development, whereas it is maximum in mid-development. The latter is, however, lower than its counterpart in eggs of *R. prolixus* (Beament, 1949). The only difference which the epembryonic membrane has from that of *P. brassicae* is the incorporation of a little unsaturated-lipoid in it with age of the eggs, which serves to some extent as a secondary waterproofing mechanism. A re-examination of this membrane of *P. brassicae* now has indicated that as development advances it also incorporates a little quantity of unsaturated-lipoid which escaped detection earlier. Moreover Beament (1949) and Matthee (1951) have also reported the formation of a secondary waterproofing mechanism in developing eggs of *R. prolixus* and *L. pardalina* respectively. In both these cases the secondary wax material is secreted by serosal cells, impregnating the secondary egg-membrane in the former egg, and giving a continuous film between the yellow and white cuticles in the latter egg. The epembryonic membrane of eggs of *T. evanescens minutum* and *T. pyrillae* is free from lipid. Further in eggs of the parasitic and the host insects it is ultimately reabsorbed by the developing larva sometimes before hatching.

It may be inferred from the investigation that all layers of the endoparasitic eggs and only the cement and chorion of eggs of the other insects do not obstruct the penetration of hydrophilic or lipophilic chemicals. The lipid layer of eggs of the other insects gives the main resistance to the entry of water-soluble materials alone, and the lipophilic substances cross this barrier quite easily. In this respect the results corroborate the earlier findings on eggs of *P. brassicae*. The appearance of oily traces in the epembryonic membrane in mid-development as well as the conversion of some unsaturated-oil of the lipid layer to saturated-state at this time may be correlated with comparatively increased resistance to desiccation and penetration of water-soluble poisons into the eggs. Such conditions are not expected to obstruct the penetration of lipophilic substances, but some resistance offered to them and to a fumigant-like hydrogen cyanide by the eggs at this stage cannot be explained clearly at present. However, the suggestion of Salkeld and Potter (1953), indicating that this resistance may be due to the fully developed state of epembryonic membrane, seems quite reasonable.

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## THE CHROMOSOMES OF *THELYPHONUS INDICUS* STOLICZKA

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### ABSTRACT

The diploid chromosome number of *Thelyphonus indicus* is 44 with 42 autosomes and an XY pair of sex chromosomes. The number is at variance with that in the only other species, *T. sepiaris*, whose chromosomes are known. Another interesting difference lies in the sex chromosome mechanism which is of the XY type in the Indian species while an XO mechanism has been reported in *T. sepiaris*. The X in the present species is the largest chromosome in the whole series and Y the smallest. All chromosomes are acrocentric.

While a great deal of knowledge has accumulated in recent years regarding the chromosomes of spiders, other Arachnida have not received the same attention. Of these, the sub-order Uropygi, consisting of interesting and aberrant forms deserves special mention. It includes large forms of tropical and sub-tropical distribution. Only two genera have been studied, i.e. *Thelyphonus sepiaris* by Millot and Tuzet (1934) and *Hypoconus formosus* by Warren (1939). But these accounts, and especially that by Millot and Tuzet, are so defective and incomplete, that it was felt that an Indian species, *T. indicus*, would be worth examining. This examination has revealed a number of interesting points regarding the chromosomes and their behaviour, and generally the spermatogenesis, and a brief account is given here.

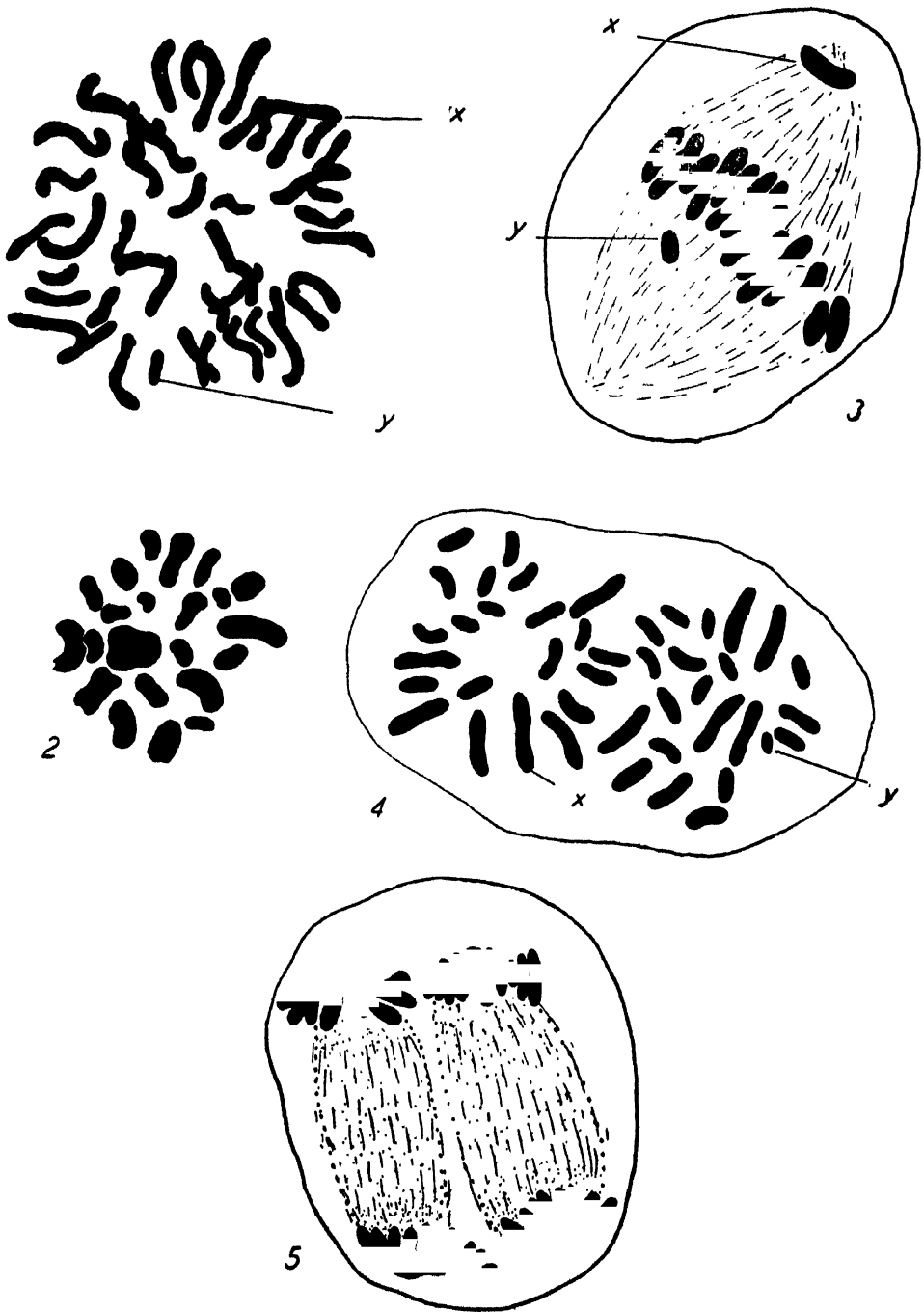
Specimens of *Thelyphonus indicus* were collected during the monsoon months of June to September around Bangalore and the testes fixed in Bouin's fluid, Carnoy's mixture and Flemming with and without acetic acid. Sections of the material were cut and stained with Heidenhain's haematoxylin and crystal-violet ; Feulgen squashes were also made.

The testes are paired convoluted tubes extending through the abdomen on either side of the ventral nerve cord. Each tube is oval in cross section and, when active, shows cells in practically all stages of spermatogenesis, the earlier stages being found in the periphery and the later ones, along with the sperms, in the centre. A full study can therefore be made of the entire process.

Spermatogonia occur as isolated cells in the periphery of the tubule and examination of their division stages reveals that the chromosome number in them is 44. Analysis of the chromosomes shows that (a) all chromosomes are acrocentric, (b) the sex complement consists of a pair of dissimilar chromosomes with X as the largest chromosome and Y the smallest. The sex chromosomes always appear to lie at the periphery of the gonial metaphase plate.

The spermatogonia pass through far fewer mitotic divisions than in most Arachnida ; perhaps 2 to 3 divisions alone occur. No evidence of any grouping of the descendants of the original spermatogonium to form cysts was seen, the various meiotic stages being distributed in a more or less haphazard manner in the tubule. A pronounced polarization of the chromosomes is seen, both in the leptotene as well as in the pachytene stages.

Diakinesis reveals 22 bivalents, 21 formed of the autosomes and one of the XY pair. The latter can be easily recognized by their behaviour and movement



TEXT-FIG. 1

Fig. 1.—Metaphase plate of spermatogonium.  $\times 4500$ .

Fig. 2.—Metaphase-I showing the bivalents.  $\times 4500$ .

Fig. 3.—Segregation of the X and Y chromosomes.  $\times 4500$ .

Fig. 4.—Metaphase plate of second meiotic division.  $\times 4500$ .

Fig. 5.—Two spindles lying in a common cell matrix.  $\times 4500$ .

on the spindle also. Both X and Y move toward the poles earlier than the autosomes and even there, X reaches its pole earlier than Y does its pole.

One of the interesting features of spermatogenesis is the precocious second meiotic division. It has been noticed as a regular phenomenon that the two second division spindles lie in a single cell matrix. The two spindles as well as their components are clear and distinct but apparently there has not been a sufficient interval between the first meiotic division and the second, for the cytosome to divide and constitute the secondary spermatocytes.

A closer examination reveals that there is practically no interphase between the two meiotic divisions and no sooner the first division is completed, than the two chromosome groups are incorporated into the two spindles of the second division. Sometimes the two spindles lie parallel in the cell matrix but often they lie without any special orientation. Apparently the second division is completed in this position and instances where the four spermatid nuclei are found in the same cytoplasmic matrix are quite common, the cytosomal division taking place soon after. But more often, the cytosomal division takes place slightly earlier, resulting in binucleate cells. In any case, this precocious second division does not seem to affect spermatogenesis in any manner, for sooner or later cytosomal division follows and uninucleate spermatids result. We have never found abnormal spermatids of any kind. Tripolar spindles are often encountered but they are probably abnormalities and do not lead to normal cells. During spermiogenesis the nucleus of the spermatid condenses. It later becomes bell shaped and spirally twisted to form the characteristic sperm head of the animal.

#### DISCUSSION

*Thelyphonus sepiaris* and *Hypoconus formosus* are the only species in the sub-order Uropygi (Arachnida) whose chromosomes have been studied so far. Millot and Tuzet (1934) described 24 chromosomes and one heterochromosome as making the haploid number in *Thelyphonus sepiaris* while Warren (1939) counted only 12 chromosomes in *Hypoconus formosus*. In the latter species, according to the author, all chromosomes seem to fuse into a single large homogeneous ring at metaphase which splits transversely into two half loops at anaphase and later condense into oval masses moving to the two poles. It is difficult to understand this phenomenon in the light of modern views on chromosome structure and morphology and a clear evaluation of the significance of these findings must await a re-examination of the chromosomes of this organism by modern methods. The description of the chromosomes of *Thelyphonus sepiaris* is more easily understood but the authors (Millot and Tuzet, 1934) admit to the possibility of one or two errors in regard to their number.

It is interesting that the number and behaviour of the chromosomes in the Indian species of *Thelyphonus* should be so different from those of *T. sepiaris*. The diploid number here is 44 with two clearly defined and dissimilar sex chromosomes which have been identified as X and Y. The chromosomes are all acrocentric and distinct at all stages and do not show the fusion described for *Hypoconus formosus* by Warren (1939).

This is the first account of XY type of sex chromosome mechanism in the Uropygi. The account by Millot and Tuzet would lead one to believe that it is of the XO type in their species of *Thelyphonus* while in *Sarax sarawakensis* (Amblypygi), the same authors describe an XO mechanism. It is too early to assess the significance of this difference and until we have information on more species of this sub-order, any general conclusions regarding the evolution of chromosomes within the group would be premature.

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# MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN *UROMYCES HOBSONI* VIZE

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## ABSTRACT

In the present study two strains of *U. hobsoni* have been distinguished: the binucleate form infecting *Jasminum grandiflorum* L. and the uninucleate form infecting *J. malabaricum* Wt. The latter has been designated as *U. hobsoni* forma *uninucleatum*. Both the strains are host specific. In the binucleate strain pycnia are regularly formed and the mode of pycniospore formation and separation has been found to be of a novel type. The structure of the telia which, in both the strains develop within old aecia, has been described in detail. The teliosporic basidia in the binucleate strain may be 2-, 3-, or 4-celled. It has been pointed out that the variability of the basidia with respect to the number of cells and nuclei in them is not an environmentally induced phenomenon. Basidiospores are bi- or quadri-nucleate. The latter are bigger in size. In the *uninucleatum* form pycnia have been found to be absent. Teloid aecia in this strain have been observed only in some collections made from stations situated at high altitudes like Purandhar and Mahabaleshwar. It has been shown that the expression of endo-condition is possibly an inherent property of some collections only and temperature variations do not play any important rôle in changing the habit of aeciospore germination. Teliosporic basidia are invariably 2-celled and the basidial variability is not as pronounced as it is in the binucleate strain found on *J. grandiflorum*. Short cycling tendencies seem to be operative in both these strains.

## INTRODUCTION

*Uromyces hobsoni* is an "opsis" rust which has been found in India to parasitise various species of *Jasminum*, notably the following: *J. arborescens* Roxb., *J. grandiflorum* Linn., *J. malabaricum* Wt., and *J. officinale* Linn. The work of Barclay (1891) on this rust particularly with respect to life-history and aeciospore germination constitutes one of the best accounts of a tropical rust. Subsequently Ajrekar and Parandekar (1931) found that the aeciospores in the case of the rust found on *J. malabaricum* are uninucleate. Thirumalachar (1939) has worked on the cytology and life-history of *U. hobsoni* found on *J. grandiflorum*. The occurrence of nuclear migrations in the primordia of primary aecia of the rust available on *J. grandiflorum* was reported earlier by the writer (1952). Following this, an extensive study was undertaken on the mode of aeciospore germination. At the same time the observation of Ajrekar and Parandekar (1931) that the aeciospores found on *J. malabaricum* are uninucleate, was also confirmed. These studies soon indicated that occasionally the aecia are teloid, and that the aeciospores under suitable conditions of moisture and aeration germinate by producing basidiospores. This discovery of endo-condition in *U. hobsoni* has been described elsewhere (Payak, 1953). In view of such results it was thought feasible to conduct further work on the morphology and cytology of this rust. During the course of these studies it also became apparent that between the *J. grandiflorum* rust and the *J. malabaricum* rust there are not only cytological differences present but that both of them are also host specific, i.e. they are not cross inoculable. Two forms, therefore, have been distinguished: the binucleate form found on *J. grandiflorum*



and the uninucleate form found on *J. malabaricum*. The morphology and cytology of both these forms have been separately described.

#### MATERIALS AND METHODS

*J. grandiflorum* is cultivated in the gardens for its fragrant flowers while *J. malabaricum* is a wild twining bush found throughout the Western Ghat forests of India. The *grandiflorum* rust was collected locally in Poona. Studies on the *malabaricum* rust are based on material collected from Khandala, Mahableshwar and Purandhar Hill Fort.

Pycnia (in case of the *grandiflorum* rust) and aecial material was fixed in formol-acetic-alcohol and Flemming's weak fluid. Telial material was fixed in a stronger variant of Flemming's containing a higher percentage of osmic acid. Good fixation was also obtained in Gilson's fixative which has mercuric chloride as one of the main ingredients. Though its unsuitability for plant material was soon apparent, the fixation of the rust sori and mycelium was found to be excellent. Microtome sections were cut to a thickness of 7–12 $\mu$ . The sections were either stained in Heidenhain's haematoxylin or in Newton's Iodine Gentian Violet. Teliospores were germinated on slides. Basidia and basidiospores were fixed in acetic-alcohol and then stained either in aceto-carmin or in aceto-orcein.

Inoculation work was mainly conducted during the rainy season (July–September). Plants of *J. grandiflorum* were raised through "layering". The layered plants were transferred to 9" pots. As cuttings of *J. malabaricum* did not root easily, young seedlings growing under healthy bushes in the forest were brought to the laboratory and transferred to 6" pots. Majority of such seedlings survived. They were kept for observation for a week or more to check that they were free of any previous infection. These were then used for inoculation purposes. Aeciospores which had naturally fallen off from the aecial cups were used for inoculation. A spore suspension in sterile water was prepared and then sprayed on the plants. Alternatively the spores were dusted directly on the lower leaf surfaces which had been moistened before. The inoculated plants were kept in the moist chambers for at least 48 hours.

#### SYMPTOMS AND CULTURAL STUDIES

The symptoms of the *grandiflorum* rust have been fully described by Barclay (1891) and also by Thirumalachar (1939). The *malabaricum* rust also exhibits symptoms similar to those of the *grandiflorum* rust. The rust produces discrete or coalescent infections sometimes involving the whole length of the plant part concerned. Infections develop without any restriction on leaves, stems, and flowers. The rust incites considerable amount of hypertrophy and distortion of the affected parts. Aeciospore infections are orange-coloured which, with replacement by telia, in the dry season, turn dark brown to black.

Cultural studies were conducted to find out whether the *grandiflorum* rust can infect plants of *J. malabaricum* and *vice versa*. Infection studies were carried out with aeciospores. As is well known, the rust propagates itself in the favourable season through repeated generations of aeciospores. For inoculation work, aeciospores from the primary aecia associated with pycnia (in the *grandiflorum* rust) were selected. In the *malabaricum* rust it was rather difficult to select the primary aecia because so far pycnia have never been observed to occur. As controls, the aeciospores in each case were tested by inoculating them on their own hosts.

One lot of 4 potted plants of *J. grandiflorum* was inoculated on 4th September 1953. Faintly yellow spots on the leaves were discernible on 17th September

1953. Some of the aecia on these infections, when kept for long period indoors, became cylindrical and their peridia remained closed. These aecia were formed without being accompanied by pycnia. In the *malabaricum* rust also such repeating aecia alone were formed more or less within the same period. Aeciospores from *J. grandiflorum* which had been found to be viable and capable of producing infection, when cross inoculated on the *J. malabaricum* plants, gave negative results. Similarly aeciospores of the *malabaricum* rust when inoculated on the *J. grandiflorum* plants, failed to produce any infection. It thus shows that the two rusts can not be cross inoculated and there is basis to conclude that these two are separate and distinct forms of *U. hobsoni*. The rust found on *J. malabaricum* has been designated as *U. hobsoni* forma *uninucleatum*.

### THE *Grandiflorum* RUST

*Pycnia*: Ajrekar and Parandekar (1931) were unable to observe pycnia in the material of this rust available at Poona. Thirumalachar (1939) found them to be regularly present in this rust occurring at Bangalore. The writer also has been able to observe them in the Poona material. The pycnia are quite easy to spot because, when aecia have not yet developed, the hypertrophied pustules are more reddish orange than just orange in colour. It is on such reddish infections that the pycnia develop as yellowish to orange dots. Pycnia are subepidermal globose to flask-shaped, and have numerous ostiolar periphyses (Fig. 16).

*Pycniospore formation and separation*: The pycniosporophores are uninucleate and so are the pycniospores. The pycniosporophore nucleus, before the formation of a pycniospore, divides (Fig. 1). The upper daughter nucleus soon begins to migrate towards the apex. Concurrently, the pycniosporophore begins to differentiate a sub-apical constriction. The degree of conspicuousness of this constriction varies from pycniosporophore to pycniosporophore. It is very narrow in Fig. 2 where the nucleus in passing has become proportionately attenuated. The nucleus after reaching the apical part assumes its rounded form (Fig. 3). The sub-apical constriction now undergoes a process of stretching and elongation (Fig. 4). It continues to elongate and become thinner and thinner (Fig. 5) until the pycniospore gradually snaps off and thus gets freed from the parent pycniosporophore. After separation, the pycniospores lose their rounded form and become elongate and bacillariform (Figs. 6 and 7).

*Telia and Teliospores*: The telia in *U. hobsoni* are known (Barclay, 1891 Thirumalachar, 1939) to develop within old aecia. Though the phenomenon of formation of telia in old aecia is very well known in several rusts, particularly inopsis forms (Jackson, 1931), the morphology of such composite sori in which the two spore forms get telescoped, does not seem to have been worked out fully. Old aecia, as observed in *U. hobsoni*, have a persistent peridium and the aecial cups are either devoid of aeciospores or they may contain degenerating mass of old aeciospores which failed to get an exit from the sori. Teliospore formation is preceded by the development of numerous thick-walled hyphae. These adhere laterally and also develop one over the other in vertical chains. Ultimately the cells in mass assume an aspect of a compact cellular crust. The crust cells are at first hyaline and thin-walled but subsequently they become brown-coloured and thick-walled. A representative of such hyphae of the crust is shown in Fig. 8. Its *in situ* location is shown in Fig. 18 (arrow). Septum formation in the upper cell has not yet commenced. Curiously the lower cell is uninucleate. As can be seen in Figs. 9, 17 and 18, the uninucleate and binucleate hyphal cells of the crust occur in an intermixed condition without any order or sequence. Teliospores develop over the surface of the crust. Nuclear fusions have been observed to occur in the young teliospores regularly. Teratological teliospores like those shown in Figs. 14 and 15 occur quite commonly. In the teliospore of Fig. 14 two nuclear

spaces can be observed while in that of Fig. 15 even though the spore body has been wedged in by an invagination, only one nuclear space is present.

**Nuclear fusion:** In Fig. 10 two nuclei have just adpressed together. In Fig. 11 a partial intermingling of the chromatin has commenced but the nucleoli have still remained unfused. In Fig. 13 the fusion nucleus shows a chromatin reticulum clearly. The nucleolus has diminished considerably in size. In Fig. 12 the chromatin threads have assumed a bipartite appearance not unlike that observed during zygotene or pachytene of meiosis. In Fig. 19 is shown a two-celled teliospore. Each cell here contains a fusion nucleus. On the whole one meets two-celled teliospores in the sori of *U. hobsoni* only occasionally.

**Basidia and Basidiospores:** Maximum teliospore germination can be secured when the Southwest Monsoon is about to terminate sometime in September. The teliospores at this time mature early, germinate, and the resulting basidiospore infections produce fresh pycnia and aecia on young leaves and twigs. Teliospores produced late in the season in November or December remain dormant until the onset of the next Monsoon. Collections in winter, therefore, do not show a high percentage of teliospore germination.

Owing to scarcity of good division stages meiosis has not been studied in detail. Attention has been confined to the study of the variability in the basidial cell numbers and the nuclei that they contain—a variability which has been repeatedly noted in all the material studied so far. In Fig. 20, the fusion nucleus has migrated in the middle of the basidium. The interphasic nucleus is showing a well developed chromatin reticulum along with a nucleolus. Occasionally basidia get prematurely divided into two cells without being accompanied by a nuclear division. Such a stage is shown in Fig. 21. The region below the septum is being interpreted as a cell of the basidium and not a basidial stipe. The latter, unlike the present case, is always found to be an empty space. In Fig. 22 is shown an intranuclear spindle in side view. Chromosomes are rather irregularly distributed. In Fig. 23 the nucleus is in late anaphase I while Fig. 38 represents telophase I. The spindle in the basidium of Fig. 38 owing to the typical curvature of the latter might have been pushed aside near the wall on the right. Consequently, the telophasic stage also has continued to remain in the same parietal location as the spindle.

The daughter nuclei resulting from first meiotic division undergo second division so rapidly that they have never been observed in the interphasic state. If at all they exist in interphase it must be lasting for a very brief duration indeed. In Fig. 27 the nuclei are in anaphase II. As yet even the first septum in the basidium has not developed. In Fig. 28 the nuclei are in telophase II and the first septum formation has now become apparent. The subsequent stage—a normal 4-celled basidium—is shown in Fig. 32. The development of the basidium outlined above may be considered as typical in the sense that it is the expected sequence of occurrence following teliospore germination in any rust. In more than 50 stained preparations of germinating teliospores belonging to the *grandiflorum* rust many abnormal features of the basidia have been observed. The variability of the basidia with respect to their cell numbers and nuclear distribution, following meiosis, is considerable.

In Fig. 24 the fusion nucleus has undergone both the meiotic divisions without being accompanied by septum formation in the basidium. Completion of meiosis without wall formation in the basidium has been reported by Olive (1943) to occur occasionally in *Septobasidium apiculatum* Couch and commonly in *S. grandisporum* Couch. In the latter species each basidium produces one large-sized basidiospore.

Some of the unusual variations are those shown in Figs. 40, and 41. In Fig. 40 the basidium has become forked at the mouth of the parent teliospore. The uppermost cell of the basidium has produced an apical basidiospore. In Fig. 41 a binucleate basidiospore is developing at the junction of the basidium and the teliospore; another basidiospore is forming on the second cell from below.

Two-celled basidia are depicted in Figs. 34 and 36. Both are noteworthy in that their upper cells have produced 2 basidiospores each. In Fig. 36 the nucleus of the upper cell has remained undivided. In Fig. 34 one basidiospore developing on a side-sterigmata is binucleate while the apical basidiospore shows one nucleus and two extra-nuclear stainable granules in the cytoplasm. The nucleus in the lower cell has not divided.

Three-celled basidia occur quite frequently. The various types of such basidia have been shown in Figs. 25, 26, 29 and 39. In the basidia of the type shown in Fig. 35 it is difficult to decide about the exact number of cells that they possess. The difficulty hinges on the lower region of the basidium because it can not be decisively interpreted either as a basidial cell or as a basidial stipe. In any case, it seems certain that the nucleus has undergone meiosis in the upper cell. Two daughter nuclei migrated to the basidiospore while the rest have remained in the parent cell. Majority of the 3-celled basidia are either of the type shown in Fig. 29 or of that shown in Fig. 39. More commonly the middle cell is binucleate. The basidiospores produced from such binucleate cells are quadrinucleate while the remaining uninucleate cells give rise to binucleate basidiospores. The latter are shorter in size (in stained preparations they measure  $10.5-16.5 \times 4.5-6.0\mu$ ), while the quadrinucleate basidiospores on the same basidia are proportionately bigger in size ( $18.0-22.5 \times 6.0-7.5\mu$ ). Another interesting occurrence in the teliosporic basidia of this rust is the absence of nuclei in certain cells. These have been shown in Figs. 26, 30, 31, 33 and 43. In Fig. 37 a quadrinucleate discharged basidiospore has been depicted. The basidium of Fig. 42 has produced 3 basidiospores. Two nuclei are apparent in each of the two lower basidiospores. In the upper one, one of the nuclei is dividing.

#### THE UNINUCLEATUM FORM

An endo-condition was demonstrated in a collection of this rust made from Purandhar Hill fort (Payak, 1953). Further work has shown that the aeciospores of this rust available at Mahableshwar also germinate by producing 2-celled basidia. However, in the population of the rust extant at places like Khandala, endo-condition was found to be absent. Aeciospores from such localities almost always produced 2-celled germ tubes with whip-like extensions instead of the basidiospores. Arthur (1929) has pointed out that short-cycling tendencies are more apparent at higher altitudes and deeply sheltered valleys where the atmospheric conditions are undoubtedly different from those prevailing in the plains. In order to find out whether basidiospore production is an expression of temperature response, fresh aeciospores collected from Khandala, were kept in moist chambers in a refrigerator at temperatures varying from  $18^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ . Precautions were taken to see that the developing basidia from the aeciospores did not get covered up by the condensing moisture. Even in an incubation at such low temperatures basidiospores failed to develop. Aeciospores from Mahableshwar, however, regularly produced basidiospores even at room temperatures ( $28^{\circ}\text{C}$  to  $30^{\circ}\text{C}$ ). It thus becomes manifest that the expression of the endo-condition is possibly an inherent property of some collections only and temperature variations do not play any important rôle in changing the habit of aeciospore germination.

*Telia and Teliospores:* The development of telia in old aecia in the opsis rusts is undoubtedly due to the suppression of the uredial stage. It may be

pointed out that occasionally telia in *U. hobsoni* do develop separately. Whether teliospores are formed in old aecia or in independent, telia, the sori are always deep seated in the form of globose sunken cavities. Teliospore formation in a separate telium is shown in Fig. 44. Here all the thin-walled cells of the hymenium are uninucleate.

Teliospores begin to form when the dry season is setting in. At this stage practically all the aeciospores have been shed away from the sori. By repeated divisions the hymenium forms vertical rows of 3-6 cells which adhere laterally. As the development progresses, the whole cell mass assumes an aspect of a parenchymatic crust or plate. The cells of this crust are at first thin-walled and hyaline. The cellular plate by further divisions rapidly elongates and comes to occupy more than half the space in the sorus. In some aecia, aeciospores which failed to escape now get compressed by the cellular crust and they get concentrated in the central region of the sorus (Fig. 45). This entire mass ultimately undergoes dissolution giving way to the developing teliospores.

In Fig. 46 a telium is shown which has arisen as a separate sorus. The cells of the hymenial plate are hyaline and thin-walled. In Fig. 47 the sorus, unlike that of Fig. 45, is devoid of old aeciospores. The thickening and browning commences in the intercellular hyphae which converge at the base of the sorus progressing upwards in the sorus until in the mature telium the entire cellular crust becomes hard. The thick-walled cells of the crust remain uninucleate throughout (Fig. 47). Ordinarily the cellular plate, as mentioned above, occupies about half the soral space but sometimes it assumes a domelike aspect and fills up practically the entire soral cavity (Fig. 48).

Since the crust cells are uninucleate from the beginning the resulting pedicellate teliospores also individually contain only one nucleus. One such teliospore has been shown in Fig. 49. As the binucleate condition in the constituents of young as well as mature telia is conspicuous by its absence, nuclear fusion does not occur in the teliospores of this form.

**Basidia and Basidiospores:** Teliospore germination, in the collections of the *uninucleatum* rust made at different places at various times, has been so erratic and in such low percentage that inoculation work with teliospores has been so far unsuccessful. However, a few stages of teliospore germination observed so far have provided some understanding of nuclear cytology of the basidium. Basidia are invariably two-celled. The nucleus from the teliospore migrates in the middle of the basidium (Fig. 50), divides (Fig. 51), and the daughter nuclei get distributed in the two cells of the basidium (Fig. 52). Occasionally the teliosporic nucleus in the basidium does not divide even though the latter becomes two-celled (Fig. 53). The nucleus in the lower cell is about to migrate in the basidiospore on the left. The occasional presence of such configurations demonstrates from a cytological point of view the truly haploid nature of this strain.

The basidia produce only two basidiospores. The nuclei after migration from the basidial cells divide once more (Figs. 55, 56 and 57). The primary basidiospores sometimes produce secondary ones (Fig. 54). The secondary basidiospores also, like the primary ones, are formed on well developed sterigmata. Thirumalachar (1939) has observed the formation of even tertiary basidiospores in the *grandiflorum* rust. A germinating basidiospore with both its nuclei intact (i.e. without degeneration) in the germ tube has been shown in Fig. 58. A comparison of aeciospore and teliospore germination within the *uninucleatum* form itself demonstrates the remarkable similarity between aeciosporic basidia and the teliosporic basidia. Both types are invariably composed of 2 uninucleate cells and both of them produce two binucleate basidiospores.

As stated above, so far pycnia have never been found to occur in this rust. Teliospore germination being very scanty, evidence through inoculation work

about the actual occurrence or nonoccurrence of pycnia, is, at present, not available. Intensive field observations, especially at the commencement of the rainy season when the rust begins to appear in the forests, have so far failed to reveal the presence of pycnia.

### DISCUSSION

The mode of pycniospore formation in the rusts whose pycnia and their constituents have been rather well investigated, deserves comparison with that found in the *grandiflorum* rust. Blackman (1904) first indicated that the free ends of the pycniosporophores in *Gymnosporangium clavariaeforme*, are provided with subapical thickened rings. Presumably the separation of the newly-formed pycniospore is accomplished through this thickened ring. A somewhat similar mode of pycniospore separation has been described by the writer in *Scopella gentilis* (Payak, 1956). Olive (1944) found that in *Gymnosporangium clavipes* instead of thickened rings, pycniosporophores have open collars through which pycniospores are budded out. Colley (1819) in *Cronartium ribicola*, and Lamb (1934) in *Puccinia prostii* have shown that pycniosporophores form pycniospores through subapical constrictions. In the latter rust the pycniospore gets cut off by a cell wall. The subapical constrictions develop in the pycniosporophores of the *grandiflorum* rust also. But the separation is achieved by the gradual narrowing and attenuation of the constriction until the pycniospore frees itself from the pycniosporophore.

Jackson (1935) has reviewed the then known basidial variations in rusts and has grouped them into six types. Since then a great amount of work has been done on rusts having either an unstable life-cycle or whose basidia have a pronounced tendency to vary—both cytologically and developmentally. As Skolko (1944) has pointed out, the important consideration is whether such tendencies are fixed and typical for any given Basidiomycetous fungus, or whether they are mere fortuitous occurrences. If it is the latter then no great biological significance need be attached to them. Great as is the capability of a heterobasidiomycete basidium to vary, Rogers (1934) has emphasised the need for cautious interpretation of atypical forms in the following words: "not only is there great variation in basidial morphology from group to group and species to species, but under suboptimum conditions the heterobasidiomycete basidium is capable of any of the modifications possible to ordinary mycelium—indefinite elongation, repeated irregular septation, branching, oidium formation—and of direct germination, by hyphae instead of by basidiospores, in their occasional or regular septation and their germination by repetition or conidia as well as directly. So universal is this capacity for variation, phyletic and ontogenetic, that it may well be taken to be the surest criterion of the group; and the types of response possible must be taken into account in any attempt at interpretation of the more aberrant." In the *grandiflorum* rust, the variability with regard to the basidial cell numbers and nuclear behaviour becomes manifest in normal conditions, that is, conditions not far different from those obtaining in nature. The basidial variability here, therefore, seems to be an inherent capacity which expresses itself without regard to the environmental conditions. The variations noted in the *grandiflorum* strain of *U. hobsoni* are comparable to those found by Olive (1943) in various species of *Septobasidium*, and also in the rusts—*Sphenospora kevorkianii* (Olive, 1947), and *Gymnosporangium clavipes* (Olive, 1949).

Basidially bisporous strains have been found in many rusts particularly in the endo-forms. However, not all such rusts are uninucleate. Recently, Thirumalachar and Narasimhan (1950) and Thirumalachar and Govindu (1954) have described two new species of *Endophyllum*—*E. heliotropii* and *E. spilanthes* respectively which have two-celled basidia. In the former the mycelium is binucleate

and in the aeciospores the two nuclei do not fuse. Both the nuclei on aeciospore germination get distributed in each cell of the basidium and due to their subsequent division in the basidiospores, the latter become binucleate. In *E. spilanthes* the two nuclei from the aeciospores without fusion migrate to the basidium. These divide in the basidium itself but once and thus each basidial cell becomes binucleate. Basidiospores also are binucleate. There are, however, uninucleate strains known in the endo-forms as well as in the other rusts, like *Endophyllum euphorbiae-sylvaticae* var. *uninucleatum*, *E. centranthi-rubri*, *Gymnoconia nitens*, and *Uromyces rudbeckiae*. The *uninucleatum* form of *U. hobsoni* is not only haploid and basidially bisporous but also, what is more noteworthy, its aeciospores are teloid at least in some collections and they too like the teliospores develop only two-celled basidia. There is little doubt that short-cycling tendencies are operating not only in the *grandiflorum* strain but also in the *uninucleatum* form.

#### ACKNOWLEDGEMENTS

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## EXPLANATION OF FIGURES

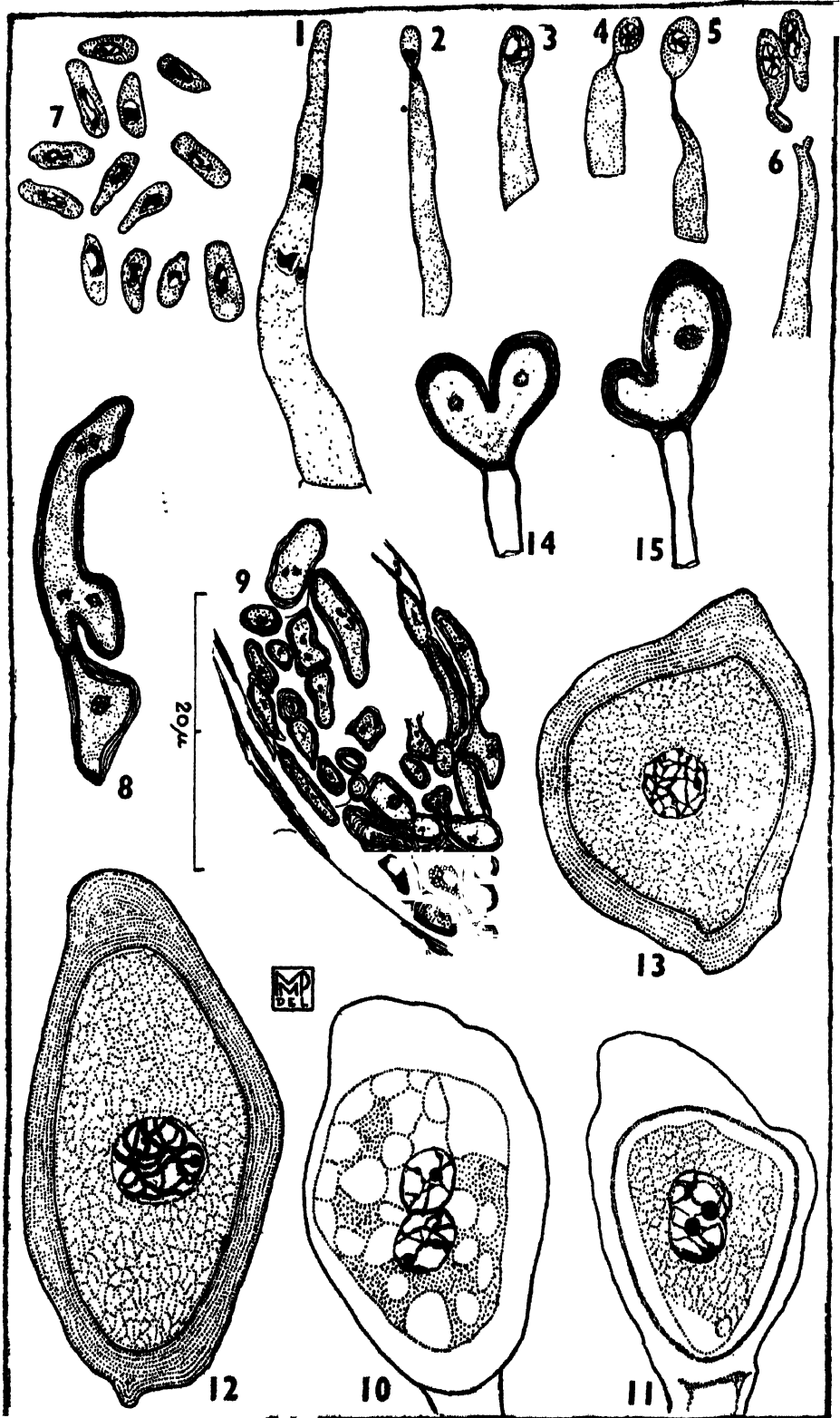
*The Grandiflorum Rust*

- Fig. 1, nucleus in telophase in a pycniosporophore before pycniospore formation,  $\times 1,800$ .  
 Fig. 2, nucleus migrating through subapical constriction of a pycniosporophore,  $\times 1,800$ .  
 Fig. 3, apical part of a pycniosporophore showing a less pronounced constriction,  $\times 1,800$ .  
 Fig. 4, subapical constriction of pycniosporophore in a state of extension,  $\times 1,800$ .  
 Fig. 5, later stage,  $\times 1,800$ .  
 Fig. 6, tip of a pycniosporophore showing scar of pycniospore separation; the latter lies just above on the left,  $\times 1,800$ .  
 Fig. 7, individual pycniospores containing elongate nuclei,  $\times 1,800$ .  
 Fig. 8, two hyphal cells from a hymenial crust in telium (cf. Fig. 18),  $\times 860$ .  
 Fig. 9, part of a telium showing mixture of uni- and bi-nucleate cells (cf. Fig. 17),  $\times 860$ .  
 Fig. 10, a teliospore showing adpressed nuclei before fusion,  $\times 1,800$ .  
 Fig. 11, later stage,  $\times 1,800$ .  
 Fig. 12, fusion nucleus in teliospore showing bipartite appearance of chromatin threads,  $\times 1,800$ .  
 Fig. 13, fusion nucleus in interphase in teliospore,  $\times 1,800$ .  
 Figs. 14, 15, two teratological teliospores,  $\times 500$ .  
 Fig. 16, a mature pycnium,  $\times 380$ .  
 Fig. 17, same as figure 9,  $\times 550$ .  
 Fig. 18, showing *in situ* location of two hyphae (arrow) sketched in Fig. 8,  $\times 740$ .  
 Fig. 19, a two-celled teliospore,  $\times 1,790$ .  
 Fig. 20, interphasic fusion nucleus in middle of basidium,  $\times 1,800$ .  
 Fig. 21, two-celled basidium; lower cell without nucleus,  $\times 1,800$ .  
 Fig. 22, Metaphase I,  $\times 1,800$ .  
 Fig. 23, Anaphase I,  $\times 1,110$ .  
 Fig. 24, 4-nucleate non-septate basidium,  $\times 1,110$ .  
 Fig. 25, 3-celled basidium, each cell uninucleate,  $\times 1,800$ .  
 Fig. 26, 3-celled basidium, note one non-nucleate cell,  $\times 1,800$ .  
 Fig. 27, Anaphase II, first septum not yet evident,  $\times 1,110$ .  
 Fig. 28, Telophase II, septum evident,  $\times 1,110$ .  
 Fig. 29, 3-celled basidium; upper cell binucleate,  $\times 1,800$ .  
 Fig. 30, 4-celled basidium; upper cell without nucleus,  $\times 1,110$ .  
 Fig. 31, 4-celled basidium; one cell uninucleate, lower cell binucleate,  $\times 1,110$ .  
 Fig. 32, 4-celled basidium; each cell uninucleate,  $\times 2,160$ .  
 Fig. 33, 3-celled basidium; middle cell without nucleus,  $\times 2,160$ .  
 Fig. 34, 2-celled basidium; upper cell producing two basidiospores,  $\times 1,330$ .  
 Fig. 35, most probably a 2-celled basidium; basidiospore binucleate,  $\times 1,330$ .  
 Fig. 36, 2-celled basidium with each cell uninucleate; upper cell with two basidiospores,  $\times 1,330$ .  
 Fig. 37, a quadrinucleate basidiospore,  $\times 1,330$ .  
 Fig. 38, Telophase I,  $\times 720$ .  
 Fig. 39, 3-celled basidium; note middle binucleate cell,  $\times 570$ .  
 Fig. 40, basidium forked at teliospore mouth,  $\times 1,090$ .  
 Fig. 41, basidiospore forming at base of basidium just above teliospore,  $\times 960$ .  
 Fig. 42, basidium with 3 basidiospores,  $\times 840$ .  
 Fig. 43, 4-celled basidium with two basidiospores; upper one quadrinucleate, lower one binucleate,  $\times 840$ .

*Forma Uninucleatum*

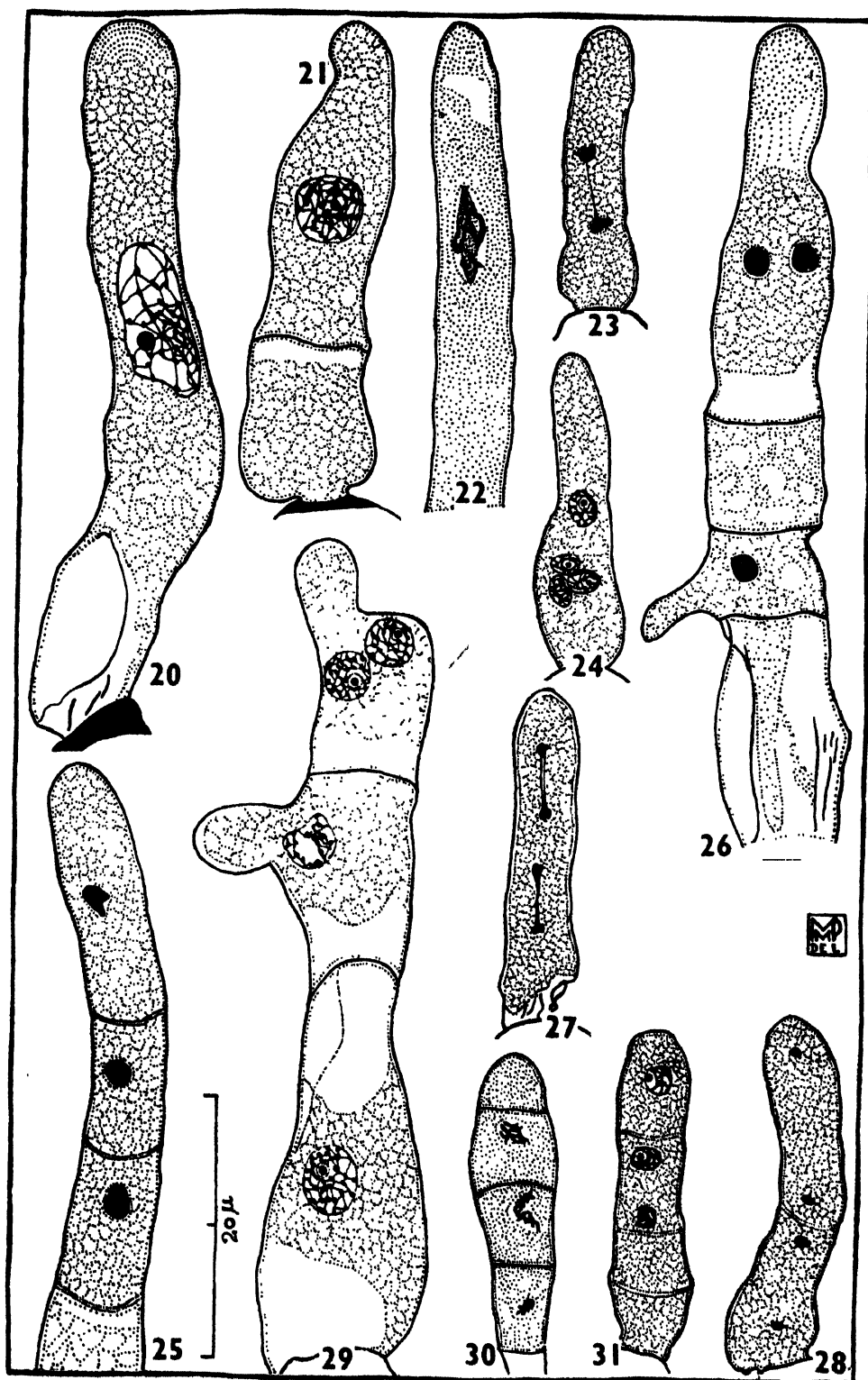
- Fig. 44, an independent telium, hymenial cells uninucleate,  $\times 270$ .  
 Fig. 45, parenchymatous crust replacing old aeciospore mass in a young telium,  $\times 200$  approx.  
 Fig. 46, cells of the crust in a telium thin-walled and hyaline,  $\times 200$  approx.  
 Fig. 47, a telium showing uninucleate hyphal cells of the crust,  $\times 270$ .  
 Fig. 48, telial crust occupying major part of sorus,  $\times 200$  approx.  
 Fig. 49, a uninucleate teliospore,  $\times 2,960$ .  
 Fig. 50, basidium with nucleus in interphase,  $\times 1,800$ .  
 Fig. 51, nucleus in telophase,  $\times 1,800$ .  
 Fig. 52, 2-celled basidium; each cell uninucleate,  $\times 1,800$ .  
 Fig. 53, 2-celled basidium; teliosporic nucleus migrating in basidiospore on left,  $\times 860$ .  
 Fig. 54, 2-celled basidium; upper basidiospore producing secondary basidiospore,  $\times 860$ .  
 Fig. 55, basidium with a binucleate basidiospore,  $\times 860$ .  
 Fig. 56, 2-celled basidium with 2 binucleate basidiospores,  $\times 860$ .  
 Fig. 57, basidium showing binucleate discharged basidiospore,  $\times 1,800$ .  
 Fig. 58, a binucleate germinating basidiospore; nuclei in the germ tube,  $\times 430$ .

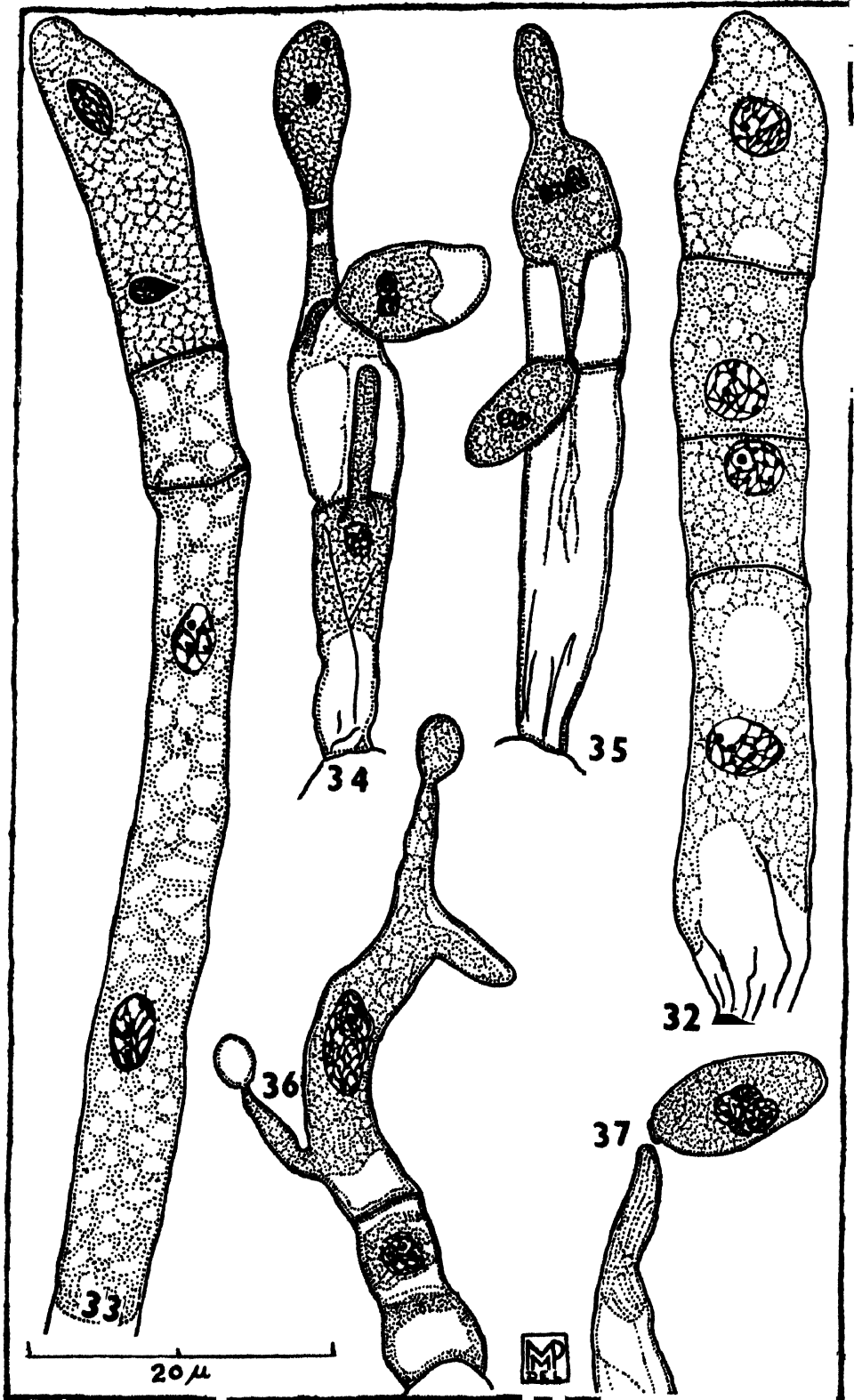






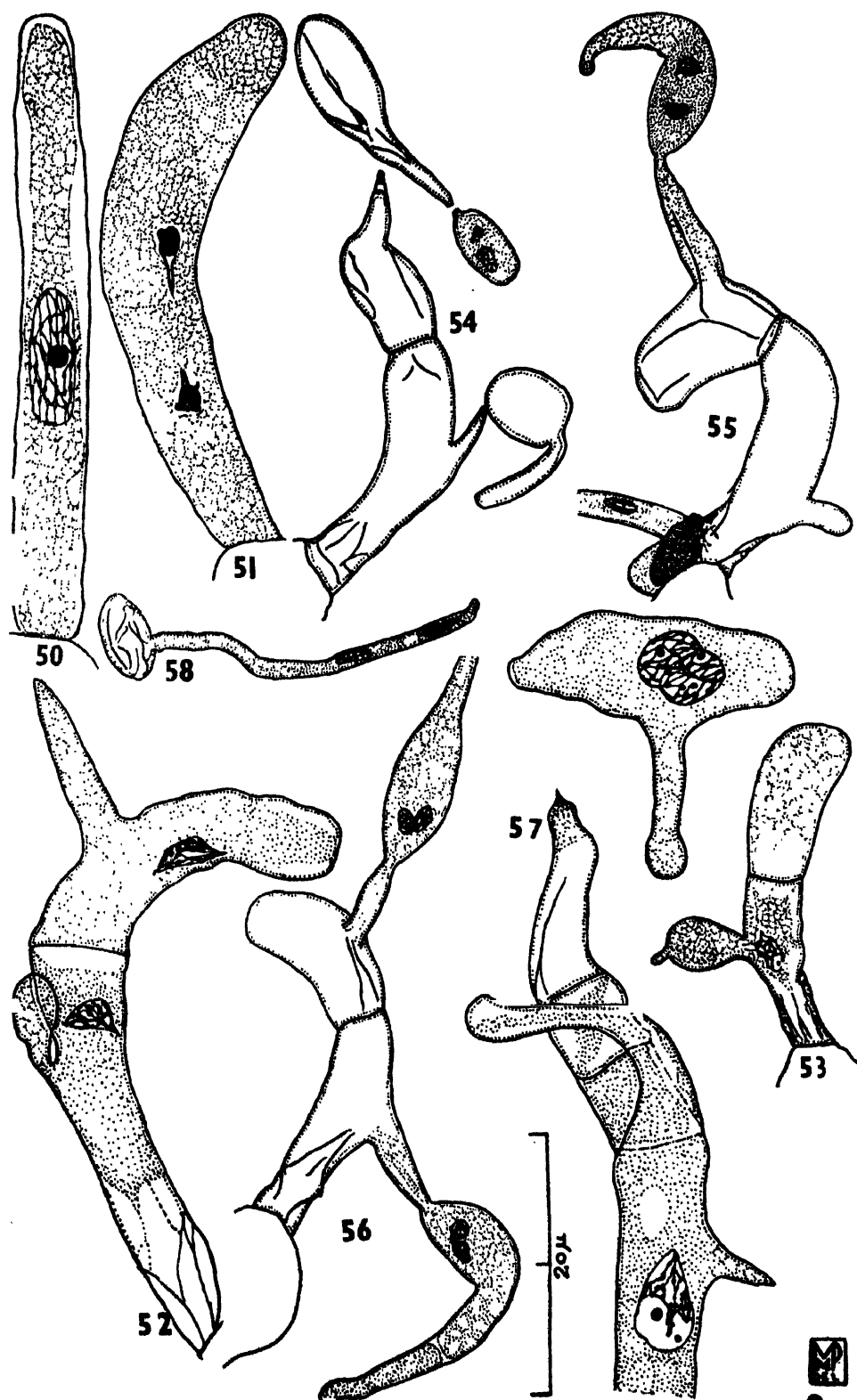














# INTERRELATION BETWEEN RESPIRATION, NET ASSIMILATION RATE, ORGANIC AND INORGANIC PHOSPHORUS UNDER PHOSPHORUS DEFICIENCY CONDITIONS

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## ABSTRACT

The respiratory index of component organs of sugarcane variety Co 453 was determined under laboratory conditions at successive stages of the life cycle in relation to phosphorus deficiency, age of the plant and the nature of the respirable materials.

Respiratory index, in general, was high during the early stage and declined towards maturity. A critical limit of sucrose (1 mgm.), glucose (0.1 mgm.), fructose (0.1 mgm.), amino acids (40 mgm.), amides (10 mgm.) and organic phosphorus (0.4 mgm.) per 100 gm. plant material helped in maintaining a high respiration rate. Further increase, however, resulted in a fall in respiration.

The possibility of high respiration being associated with high sucrose/hexose (5 : 1), and low amino/amide (3 : 1) and organic/inorganic phosphorus (8 : 1) ratios was clearly indicated. An adequate sucrose/hexose, amino acids/amide nitrogen and organic/inorganic phosphorus appeared to be the principal factors in determining the relation between age and respiratory index.

High respiratory index of leaf was associated with its high insoluble nitrogen, total nitrogen and inorganic phosphorus content. Medium respiratory index of the roots was related to medium content of total soluble and insoluble nitrogen and organic and inorganic phosphorus. Poor respiratory index of stem, on the other hand, showed some relation with low insoluble nitrogen, total nitrogen and inorganic phosphorus content.

Reasons for poor respiratory index of the stem in spite of rich respirable substrate, e.g. high sucrose, reducing sugars, amino acids, amides and organic phosphorus compounds have been discussed. Effects of phosphorus deficiency on respiration were generally insignificant.

High net assimilation rate of leaves during the early stage and relatively low N.A.R. under phosphorus deficiency were characteristically recorded. Absence of phosphorus interfered with further elaboration of amides and amino acids and thus resulted in poor protein content which was primarily responsible for low dry matter accumulation per unit area. The decline with age, on the other hand, was associated with poor chlorophyll content, reduced efficiency of leaves to absorb CO<sub>2</sub> during photosynthesis and the general decline in proteins which formed the bulk of the dry matter.

## INTRODUCTION

Supply of phosphorus in the culture medium brings about characteristic variations in the uptake of phosphorus and its further elaboration into organic phosphorus compounds. The concentration of these organic and inorganic phosphorus compounds has been suggested very often to play an important part in the respiratory processes often resulting in a marked variation in the CO<sub>2</sub> output of the plant. Phosphorus deficiency has also been shown to bring about increase in the chlorophyll content and to alter the net assimilation rate (N.A.R.). Phosphorus deficiency effects on respiration and chlorophyll content have been reported earlier by Lal *et al.* (1951, 1952). It is the intention to trace in these pages the further interrelation, if any, between respiration, organic and inorganic phosphorus content, and N.A.R. of the plant.

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## EXPERIMENTATION

The plant materials for these studies were secured from sugarcane (var. Co 453) grown under (A) complete nutrition, and (B) phosphorus deficiency conditions with the help of Hoagland's nutrient solutions as described earlier (Lal and Singh, 1957). Root, stem and leaf were carefully washed to remove dirt and adhering sand particles, chopped off separately and brought to the laboratory. The rate of respiration of these plant organs was determined by Blackman's continuous current method using  $\text{Ba}(\text{OH})_2$  as an absorbent of  $\text{CO}_2$ . The respiration measurements were taken on detached leaf, stem and root under a constant temperature of  $30^\circ\text{C}$  and in a  $\text{CO}_2$  free stream of air. The rate of  $\text{CO}_2$  output was finally expressed in mgm. of  $\text{CO}_2$  per 100 gm. of the dry matter.

Net assimilation rate of the plant at successive stages of the life cycle was determined by the formula :—

$$\text{N.A.R.} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\text{Log}_e L_2 - \text{Log}_e L_1}{L_2 - L_1} \times 1000$$

where  $W_2$  and  $W_1$  were the dry weights and  $L_2$  and  $L_1$  the leaf area at time intervals of  $T_2$  and  $T_1$ , and expressed as grams of dry matter formed per 1000 sq. cms. of leaf area per day.

The inorganic phosphorus content was determined colorimetrically by the modified Truog and Meyer method (1929) as described by Bertramson (1942). Organic phosphorus was determined indirectly by subtracting the value of inorganic phosphorus from that of total phosphorus. All observations on respiration, N.A.R., inorganic and organic fractions were statistically examined to indicate the significance of the experimental data.

## EXPERIMENTAL FINDINGS

A. *Respiratory Index :*

Respiratory index of sugarcane was found to vary to a certain extent with the plant parts, deficiency of phosphorus and with the age of the plant. Thus, in leaf, increase in age brought about marked improvement in the respiratory index which reached the highest level towards maturity. Phosphorus deficiency during early stages showed some increase in the leaf respiration but at later periods, the disparity between respiratory index of complete-nutrient and phosphorus-deficient plants was not so evident. In the stem, on the other hand, increases in age induced a marked decline in respiratory index reaching fairly low values towards maturity. This was true for both the phosphorus-deficient and complete-nutrient cultures. Barring the earliest stage of 45 days, when the complete-nutrient canes showed relatively high respiratory index of the stem in comparison to the phosphorus-deficient stem, at other stages the differences were not so characteristic. In so far as the roots were concerned, the general decline in the respiratory index of the root with age was evident, but the effect of phosphorus deficiency varied at successive samplings. During 45 and 135 days a tendency to increase respiratory index was particularly noted under phosphorus deficiency (Table IA).

Statistical analysis of the results, however, indicated the average response of the main effects and interactions to be insignificant (Table IB). The fall in the respiration rate with age also did not attain the significant level. Phosphorus deficiency too showed less marked variations in respiration. A general tendency of the leaf to exhibit higher respiration rate than that of the root which showed relatively high values in comparison to the stem, was observed (Table IC). The

TABLE I

*Effect of phosphorus deficiency on the respiratory index of sugarcane (var. Co 453) under sand nutrient cultures*

(mgm. of CO<sub>2</sub> per 100 gm. dry weight)

## A. MAIN TABLE

| Age in days | Treatments | Plant Parts |       |       | Average plant |
|-------------|------------|-------------|-------|-------|---------------|
|             |            | Leaf        | Stem  | Root  |               |
| 45          | CN         | 9.4         | 43.9  | 27.8  | 27.03         |
|             | -P         | 13.4        | 23.5  | 43.0  | 26.63         |
|             | Difference | -4.0        | +20.4 | -15.2 | +0.40         |
| 90          | CN         | 22.3        | 16.6  | 40.1  | 26.33         |
|             | -P         | 20.8        | 19.6  | 35.4  | 25.26         |
|             | Difference | +1.5        | -3.0  | +4.7  | +1.07         |
| 135         | CN         | 36.2        | 8.6   | 9.7   | 18.16         |
|             | -P         | 36.6        | 9.5   | 10.3  | 18.80         |
|             | Difference | -0.4        | -0.7  | -0.6  | -0.64         |
| 225         | CN         | 38.7        | 2.4   | 7.6   | 16.23         |
|             | -P         | 39.2        | 2.7   | 7.7   | 16.53         |
|             | Difference | -0.5        | -0.3  | -0.1  | -0.30         |

## B. ANALYSIS OF VARIANCE

| Due to                   | D.F. | S.S.    | M.S.S. | Ratio | F at 5% |
|--------------------------|------|---------|--------|-------|---------|
| Age                      | 3    | 489.92  | 163.30 | 0.56  | 8.74    |
| Treatments               | 1    | 0.11    | 0.11   | 0.00  | 24.40   |
| Plant parts              | 2    | 512.17  | 256.08 | 0.88  | 19.41   |
| Age × Treatments         | 3    | 2.57    | 0.85   | 0.00  | 8.74    |
| Treatments × Plant parts | 2    | 49.82   | 24.91  | 0.08  | 19.41   |
| Error                    | 12   | 3459.90 | 288.32 |       |         |
| Total                    | 23   | 4514.49 |        |       |         |

S.E. =  $\pm 16.98$ .

respiratory index, however, did not vary significantly with any of the conditions of the experiment (Table ID).

*Net Assimilation Rate :*

The N.A.R. of the plant was highest during the earliest stage of the life cycle and declined as age advanced. This was true of both the complete nutrient and phosphorus-deficient canes. The differences at later stages of the life cycle appeared less significant. A general tendency of lower N.A.R. under phosphorus deficiency was recorded (Table IIA). While this was true of sand nutrient cultures, the comparative data on soil cultures showed the useful effect of 60 ppm. of P<sub>2</sub>O<sub>5</sub> in improving N.A.R. This was particularly noted during the first three stages of observation, indicating thereby the possibility of greater efficiency of the plant under 60 ppm. in the accumulation of dry matter (Table IIB).

TABLE I—(Contd.)

## C. AGE × TREATMENTS INTERACTIONS

| Treatments                                      | Age in days |        |        |        | Mean of 12 values |
|---|-------------|--------|--------|--------|-------------------|
|   | 45          | 90     | 135    | 225    |                   |
| CN  | 27.03       | 26.33  | 18.17  | 16.23  | 21.9418           |
| -P  | 26.63       | 25.27  | 18.80  | 16.53  | 21.8083           |
| Mean of 6 values                                | 26.833      | 25.800 | 18.483 | 16.383 | ..                |
| C.D. at 5% for means of 4 values = $\pm 30.229$ |             |        |        |        |                   |
| " " " 6 " = $\pm 21.349$                        |             |        |        |        |                   |
| " " " 12 " = $\pm 15.096$                       |             |        |        |        |                   |

## D. TREATMENTS × PLANT PARTS INTERACTIONS

| Treatments                                      | Plant Parts |        |        | Mean of 12 values |
|---|-------------|--------|--------|-------------------|
|   | Leaf        | Stem   | Root   |                   |
| CN  | 26.650      | 17.875 | 21.300 | 21.9418           |
| -P  | 27.500      | 13.825 | 24.100 | 21.8083           |
| Mean of 8 values                                | 27.075      | 15.850 | 22.700 |                   |
| C.D. at 5% for means of 4 values = $\pm 26.159$ |             |        |        |                   |
| " " " 8 " = $\pm 18.499$                        |             |        |        |                   |
| " " " 12 " = $\pm 15.098$                       |             |        |        |                   |

TABLE II

*Effect of phosphorus deficiency on the average net assimilation rate of sugarcane plant (var. Co 453) per 1000 sq. cms. per day during successive stages of the life cycle under sand and soil nutrient cultures*

## A. SAND CULTURES

| Treatments | Age in days |      |      |      |      |
|------------|-------------|------|------|------|------|
|            | 45          | 90   | 135  | 180  | 225  |
| CN         | 1.57        | 0.48 | 0.47 | 0.15 | 0.31 |
| -P         | 1.45        | 0.39 | 0.12 | 0.25 | 0.27 |

## B. SOIL CULTURES

| Age in days | Concentration of $P_2O_5$ in ppm. of soil |       |       |       |       |
|-------------|---|-------|-------|-------|-------|
|             | 0   | 20    | 40    | 60    | 80    |
| 45          | 1.999                                     | 1.966 | 1.964 | 2.243 | 1.948 |
| 90          | 0.328                                     | 0.384 | 0.419 | 0.431 | 0.304 |
| 135         | 0.249                                     | 0.251 | 0.191 | 0.336 | 0.306 |
| 225         | 0.147                                     | 0.041 | 0.070 | 0.024 | 0.025 |

*Inorganic and Organic Phosphorus :*

The inorganic fraction of phosphorus showed slight variations with age, conditions of phosphorus deficiency and plant parts (Table IIIA). Taking the over-all plant part values into consideration, the inorganic phosphorus content did not vary significantly with age under both the conditions of nutrition (Table IIIB). The differences due to phosphorus deficiency or the plant part were equally insignificant (Table IIIC).

TABLE III

*Effect of phosphorus deficiency on the inorganic phosphorus content of sugarcane (var. Co 453) under sand nutrient cultures*

(gms. of  $P_2O_5$  per 100 gm. dry matter)

## A. MAIN TABLE

| Age in days | Treatments | Plant Parts |         |         | Average (plant) |
|-------------|------------|-------------|---------|---------|-----------------|
|             |            | Leaf        | Stem    | Root    |                 |
| 45          | CN         | 0.0527      | 0.0811  | 0.0518  | 0.0619          |
|             | -P         | 0.0437      | 0.0705  | 0.0766  | 0.0636          |
|             | Difference | +0.0090     | +0.0106 | -0.0248 | -0.0017         |
| 90          | CN         | 0.0534      | 0.0488  | 0.0828  | 0.0617          |
|             | -P         | 0.0459      | 0.0260  | 0.1001  | 0.0573          |
|             | Difference | +0.0075     | +0.0228 | -0.0173 | +0.0044         |
| 135         | CN         | 0.0790      | 0.0570  | 0.0431  | 0.0597          |
|             | -P         | 0.0941      | 0.0511  | 0.0421  | 0.0624          |
|             | Difference | -0.0151     | +0.0059 | +0.0010 | -0.0027         |
| 180         | CN         | 0.1515      | 0.0287  | 0.0317  | 0.0706          |
|             | -P         | 0.1169      | 0.0258  | 0.0293  | 0.0573          |
|             | Difference | +0.0346     | +0.0029 | +0.0024 | +0.0133         |
| 225         | CN         | 0.0202      | 0.0483  | 0.0505  | 0.0397          |
|             | -P         | 0.0280      | 0.0549  | 0.0362  | 0.0397          |
|             | Difference | -0.0078     | -0.0066 | +0.0143 | +0.0000         |

B. AGE  $\times$  TREATMENTS INTERACTIONS

| Treatments       | Age in days |        |        |        |        | Mean of 15 values |
|------------------|-------------|--------|--------|--------|--------|-------------------|
|                  | 45          | 90     | 135    | 180    | 225    |                   |
| CN               | 0.0619      | 0.0617 | 0.0597 | 0.0706 | 0.0397 | 0.0587            |
| -P               | 0.0636      | 0.0575 | 0.0624 | 0.0573 | 0.0397 | 0.0560            |
| Mean of 6 values | 0.0627      | 0.0595 | 0.0610 | 0.0639 | 0.0396 |                   |

C.D. at 5% for means of 3 values =  $\pm 0.4673$

" " " 6 " =  $\pm 0.3300$

" " " 12 " =  $\pm 0.2088$

TABLE III—(Contd.)

## C. TREATMENTS × PLANT PARTS INTERACTIONS

| Treatments                                      | Plant Parts |        |        | Mean of 15 values |
|---|-------------|--------|--------|-------------------|
|   | Leaf        | Stem   | Root   |                   |
| CN  | 0.0714      | 0.0528 | 0.0520 | 0.0587            |
| -P  | 0.0657      | 0.0457 | 0.0569 | 0.0560            |
| Mean of 10 values                               | 0.0685      | 0.0492 | 0.0544 | —                 |
| C.D. at 5% for means of 5 values = $\pm 0.3615$ |             |        |        |                   |
| " "   | " 10 "      | " "    | " "    | = $\pm 0.2603$    |
| " "   | " 15 "      | " "    | " "    | = $\pm 0.2088$    |

The organic fraction of phosphorus showed a definite fall with age and varied characteristically with the factors under investigation (Table IVA). The age effect and effects of plant parts were found to be statistically significant. The significant interaction between age and treatments indicated that the effects of phosphorus deficiency on the organic phosphorus content of the plant varied markedly with age (Table IVB). The over-all plant part values indicated significant rise in the organic phosphorus content of the complete-nutrient plants at 135 days and a significant fall at later periods of the life cycle. Under phosphorus deficiency, highest organic phosphorus content at 90 days and a significant decline at 225 days were also noted. The over-all age values, however, did not show any significant reduction in organic phosphorus, although a tendency of such a nature was recorded (Table IV C). A significant rise in the organic phosphorus

TABLE IV

*Effect of phosphorus deficiency on the organic phosphorus content of sugarcane (var. Co 453) under sand nutrient culture*

(gms. of  $P_2O_5$  per 100 gm. dry matter)

## A. MAIN TABLE

| Age in days | Treatments | Plant Parts |         |         | Average (plant) |
|-------------|------------|-------------|---------|---------|-----------------|
|             |            | Leaf        | Stem    | Root    |                 |
| 45          | CN         | 0.2759      | 0.4569  | 0.4846  | 0.4057          |
|             | -P         | 0.3277      | 0.5479  | 0.3970  | 0.4242          |
|             | Difference | -0.0518     | -0.0910 | +0.0876 | -0.0185         |
| 90          | CN         | 0.4558      | 0.3824  | 0.6181  | 0.4854          |
|             | -P         | 0.5452      | 0.5463  | 0.8706  | 0.6540          |
|             | Difference | -0.0894     | -0.1639 | -0.2525 | -0.1686         |
| 135         | CN         | 0.9108      | 0.4320  | 1.3217  | 0.8882          |
|             | -P         | 0.3964      | 0.3770  | 0.5359  | 0.4331          |
|             | Difference | +0.5244     | +0.0550 | -0.2142 | +0.4551         |
| 180         | CN         | 0.1152      | 0.2304  | 0.4551  | 0.2669          |
|             | -P         | 0.1472      | 0.3389  | 0.2992  | 0.2618          |
|             | Difference | -0.0320     | -0.1088 | +0.1559 | +0.0051         |
| 225         | CN         | 0.0892      | 0.1700  | 0.0729  | 0.1106          |
|             | -P         | 0.0471      | 0.0360  | 0.2754  | 0.1195          |
|             | Difference | +0.0421     | +0.1340 | -0.0227 | -0.0089         |

TABLE IV—(Contd.)

## B. ANALYSIS OF VARIANCE

| Due to                   | D.F. | S.S.   | M.S.S. | Ratio | F at 5% |
|--------------------------|------|--------|--------|-------|---------|
| Age                      | 4    | 1.1837 | 0.2959 | 11.64 | 3.01    |
| Treatments               | 1    | 0.0204 | 0.0204 | 0.80  | 24.60   |
| Plant parts              | 2    | 0.2469 | 0.1234 | 4.85  | 3.63    |
| Age × Treatments         | 4    | 0.3290 | 0.0820 | 3.22  | 3.01    |
| Treatments × Plant parts | 2    | 0.0303 | 0.0151 | 0.59  | 19.43   |
| Error                    | 16   | 0.4071 | 0.0254 |       |         |
| Total                    | 29   |        |        |       |         |

S.E. per =  $\pm 0.16$ .

## C. AGE × TREATMENTS INTERACTIONS

| Treatments                                      | Age in days    |        |        |        |        | Mean of 15 values |
|---|----------------|--------|--------|--------|--------|-------------------|
|   | 45             | 90     | 135    | 180    | 225    |                   |
| CN  | 0.4058         | 0.4854 | 0.8882 | 0.2669 | 0.1106 | 0.4313            |
| -P  | 0.4242         | 0.6540 | 0.4364 | 0.2618 | 0.1195 | 0.3791            |
| Mean of 6 values                                | 0.4150         | 0.5697 | 0.6623 | 0.2643 | 0.1150 | ..                |
| C.D. at 5% for means of 3 values = $\pm 0.2771$ |                |        |        |        |        |                   |
| “ “ “ 6 “                                       | = $\pm 0.1967$ |        |        |        |        |                   |
| “ “ “ 12 “                                      | = $\pm 0.1237$ |        |        |        |        |                   |

## D. TREATMENTS × PLANT PARTS INTERACTIONS

| Treatments                                      | Plant Parts    |        |        | Mean of 15 values |
|---|----------------|--------|--------|-------------------|
|   | Leaf           | Stem   | Root   |                   |
| CN  | 0.3694         | 0.3343 | 0.5904 | 0.4313            |
| -P  | 0.2927         | 0.4756 | 0.4756 | 0.3791            |
| Mean of 10 values                               | 0.3311         | 0.5830 | 0.5330 | ..                |
| C.D. at 5% for means of 5 values = $\pm 0.2142$ |                |        |        |                   |
| “ “ “ 10 “                                      | = $\pm 0.1542$ |        |        |                   |
| “ “ “ 15 “                                      | = $\pm 0.1237$ |        |        |                   |

content of the stem and the root over that of the leaf was also recorded. It, therefore, became obvious that the organic phosphorus content varied most characteristically with the age and plant parts and showed more or less similar trend of variations with age as noted for respiration rate. The plant parts, however, showed differential response. Thus, the leaf showed highest respiration rate followed by root and stem, but the organic phosphorus content varied in the decreasing order, stem > root > leaf.

## DISCUSSION

The data recorded in these pages indicate beyond doubt that leaves in general, showed a relatively higher rate of respiration than root or the stem, particularly during the second half of the life cycle. During early stages the roots showed higher respiratory index than the leaf. Such variations in the respiration rate

of the component organs must necessarily be analysed in terms of the important changes in the concentrations of various respirable materials. Taking the plant as a whole, the decline in respiration with age depicted, in general, the low state of metabolic activity of growing plant towards maturity, in comparison to the early stages when growth, differentiation of tissues and other metabolic changes were taking place with rapidity. Attempts have, therefore, been made to correlate the rate of respiration at various stages of the life cycle under phosphorus deficiency and complete nutrition conditions with the changes taking place in the relative concentration of various organic fractions.

When the rates of respiration were plotted against the corresponding values of carbohydrates and other organic nitrogenous compounds (Fig. 1), the relationship between respiration and concentration of each of such fractions was characteristic. A general tendency of decrease in respiration with rise in sucrose, glucose, fructose and with increase in the ratios of amino/amide nitrogen, was evident. On the other hand, increase in amide, amino acids, organic phosphorus and rise in sucrose/hexose and organic/inorganic P ratios, caused increase in respiration.

At the critical limits of sucrose (1 mg.), glucose (0.1 mg.), fructose (0.1 mg.), amino acids (40 mgm.), amides (10 mgm.) and organic phosphorus (0.4 mgm.) per 100 gm., respiration was usually high. As this limit was raised, the rate of respiration did not increase but showed decline. It was equally important to note that the ratio of sucrose/hexose and amino acids/amides and organic/inorganic phosphorus, played an important rôle in regulating the intensity of  $\text{CO}_2$  output. When the concentration of organic phosphorus was high relative to its inorganic component, a high ratio of organic/inorganic phosphorus resulted in a sharp rise in respiration. On the other hand, a low ratio of organic/inorganic phosphorus resulted in a fall in the respiration. Respiration also declined with increase in amino/amide ratio, reaching low value as the ratio 6 : 1 was reached. The greater the proportion of sucrose over hexose (high sucrose/hexose ratio) the higher were the rates of respiration of the tissues. It was also clear that glucose/fructose ratio did not appear to be as important as the other three ratios in regulating respiration.

What then caused a high respiration rate during early days and poor respiration rate at later periods? The possibility of high respiration being related to high sucrose/hexose (5 : 1) and low amino/amide (3 : 1) ratio have been indicated. Another possible factor responsible for high rate of respiration, was the ratio of organic/inorganic phosphorus (8 : 1). One of the potent factors which led to high respiration during early stage, therefore, appeared to be an adequate sucrose/hexose, amino/amide nitrogen and organic/inorganic phosphorus ratios in the tissues.

The interrelation between the concentration of various organic fractions and the respiratory activity is also made clear from summarised results recorded in Table V for the component parts of sugarcane. Thus, the high rate of leaf respiration was mostly due to its high insoluble nitrogen, total nitrogen and inorganic phosphorus content. In contrast to the leaf, the roots showed medium respiration due to the medium values of total soluble and insoluble nitrogen, and medium amounts of inorganic and organic phosphorus present therein; all sugar fractions, amide and 'rest' nitrogen were poorest in roots. In comparison to both the subterranean and the green chlorophyllous foliage, the stem showed the lowest respiration. Low respiratory index of the stem was recorded in spite of the fact that the respirable material was the richest as indicated by high sucrose, total reducing sugars, total sugars, amino acids, amides, total soluble nitrogen and organic phosphorus compounds. On the other hand, low respiratory index was related to the low insoluble nitrogen, total nitrogen and inorganic phosphorus content of this organ. The protein and inorganic phosphorus content in stem being low, the respiratory index showed a fall relative to that of the leaf and the root. Further, such storage tissues like that of stem, mostly consisted of non-living cells. The



existence of a small proportion of actively metabolizing cells, and poor efficiency of such tissues to utilize the rich respirable material already present appeared to be equally important in reducing the respiratory index. There was yet another possibility of the accumulation of harmful and toxic substances in stem which inhibited the oxidation of the respirable materials to carbon dioxide stage. The

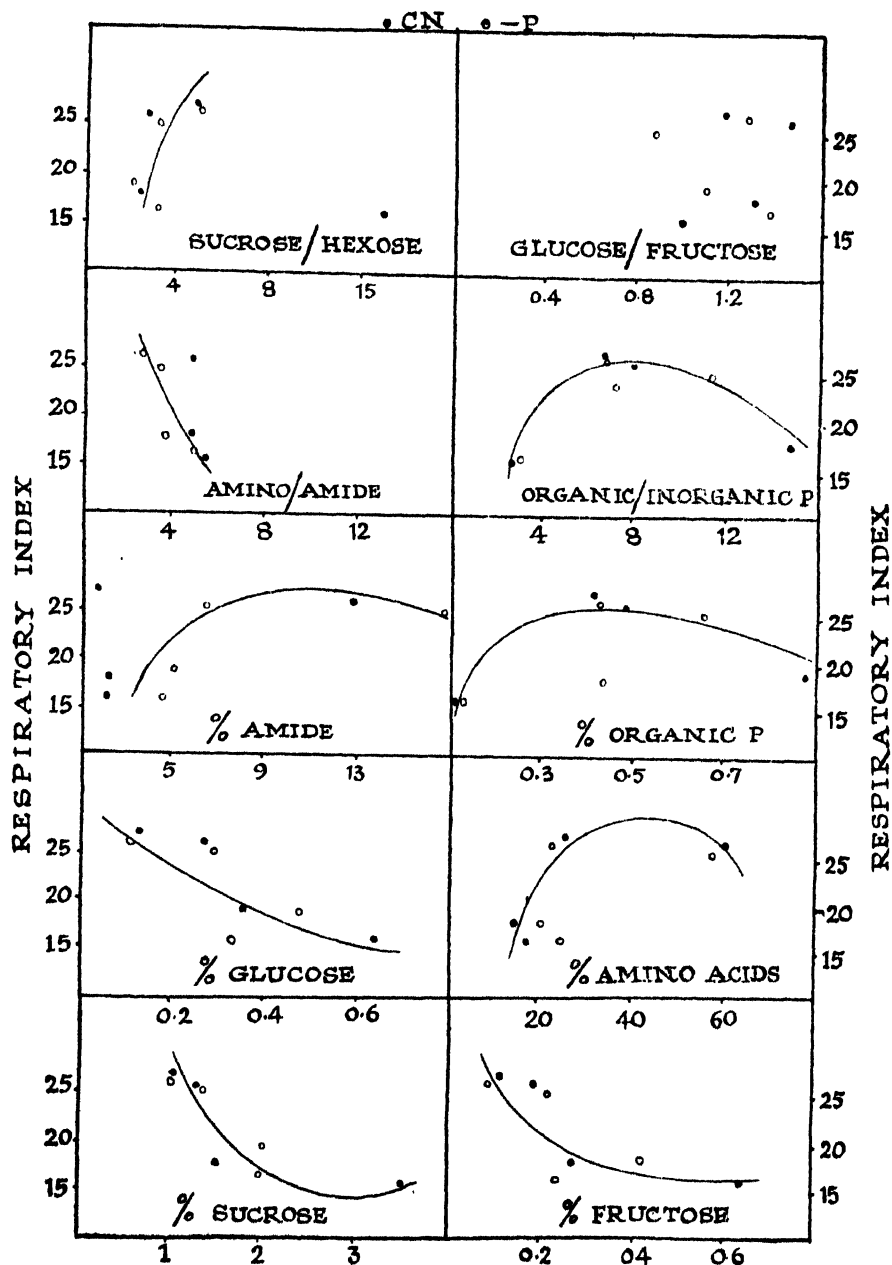


FIG. 1. Interrelation between respiration, organic and inorganic phosphorus and other fractions of nitrogen and sugars in canes grown under sand culture.

rich respirable materials present in the stem may also be converted into intermediate products such as organic acids, aldehydes and alcohol due to their restricted and incomplete oxidation caused by poor availability of oxygen, and partial narcotic effects of dissolved  $\text{CO}_2$  and other intermediate products of partial anaerobiosis. Further work is needed to elucidate the nature of this age-respiration relationship with special reference to the aerobic and anaerobic oxidation of respirable materials.

So far as the effect of phosphorus deficiency on respiration rate was concerned, relatively lower respiratory index was associated with reduction in various sugar fractions and total insoluble nitrogen and the organic and inorganic content of phosphorus in tissues. The effects of deficiency of this element on the respiratory index were generally insignificant mainly on account of the fact that possible reduction caused by the low percentage of all sugar fractions, total insoluble nitrogen and organic and inorganic phosphorus were counterbalanced by the relatively high concentration of amino acids, amides and total soluble nitrogen in phosphorus deficient tissues. If the fall in carbohydrates indicated the possibility of low index of respiration, the high amides, amino acids and total soluble nitrogen showed the possibility of higher respiratory index. Both these effects seemed to result in maintaining a balance resulting in insignificant differences in respiratory index of the canes grown in complete-nutrient and phosphorus-deficient cultures. In an analysis of respiratory drifts in sugarcane, due consideration should, therefore, be given to the relative amounts of sugar, nitrogen and phosphorus fractions present in the tissues (Table V). The possible rôle of organic acids in the respiratory activity also needed careful investigation.

TABLE V

*Order of response in component parts of sugarcane (var. Co 453) under phosphorus deficiency*

| Character                  | Order of Response  |           |
|----------------------------|--------------------|-----------|
|                            | Plant parts        | Nutrition |
| % Fructose                 | Stem > Leaf > Root | CN > -P   |
| % Glucose                  | Stem > Leaf > Root | CN > -P   |
| % Sucrose                  | Stem > Leaf > Root | CN > -P   |
| % Reducing sugars          | Stem > Leaf > Root | CN > -P   |
| % Total sugars             | Stem > Leaf > Root | CN > -P   |
| % Nitrate                  | Root > Stem > Leaf | -P > CN   |
| % Ammonia                  | Stem > Leaf > Root | CN > -P   |
| % Amide                    | Stem > Leaf > Root | -P > CN   |
| % 'Rest' nitrogen          | Stem > Leaf > Root | -P > CN   |
| % Total soluble nitrogen   | Stem > Root > Leaf | -P > CN   |
| % Total insoluble nitrogen | Leaf > Root > Stem | CN > -P   |
| % Total nitrogen           | Leaf > Root > Stem | -P > CN   |
| Respiratory index          | Leaf > Root > Stem | CN > -P   |
| Inorganic phosphorus       | Leaf > Root > Stem | CN > -P   |
| Organic phosphorus         | Stem > Root > Leaf | CN > -P   |

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# CYTOLOGY OF SOME BLECHNOID FERNS TOGETHER WITH A NOTE ON THE AFFINITIES OF *STENOCHLAENA*

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## ABSTRACT

Chromosome numbers from meiosis have been studied in the type species of three genera of the Blechnaceae. In *Blechnum orientale* and *Woodwardia radicans* the  $n$  number is 34, while in *Stenochlaena palustris* it is  $n = 73$ .

The phylogenetic affinity of the much debated genus *Stenochlaena* is discussed in the light of cumulative evidence of characters of its sporophytic and gametophytic generation. A new character of value is brought out which consists in the incurving of the margin of the fertile pinnae and its significance is discussed.

In this paper cytology of three members of the family Blechnaceae (sensu Copeland, 1947) met with in the Darjeeling Himalayas, namely *Blechnum orientale* L., *Woodwardia radicans* (L.) Smith and *Stenochlaena palustris* (Burm.) Bedd. has been investigated. There is no dispute regarding the systematic position of the first two while the last one is a problematic genus.

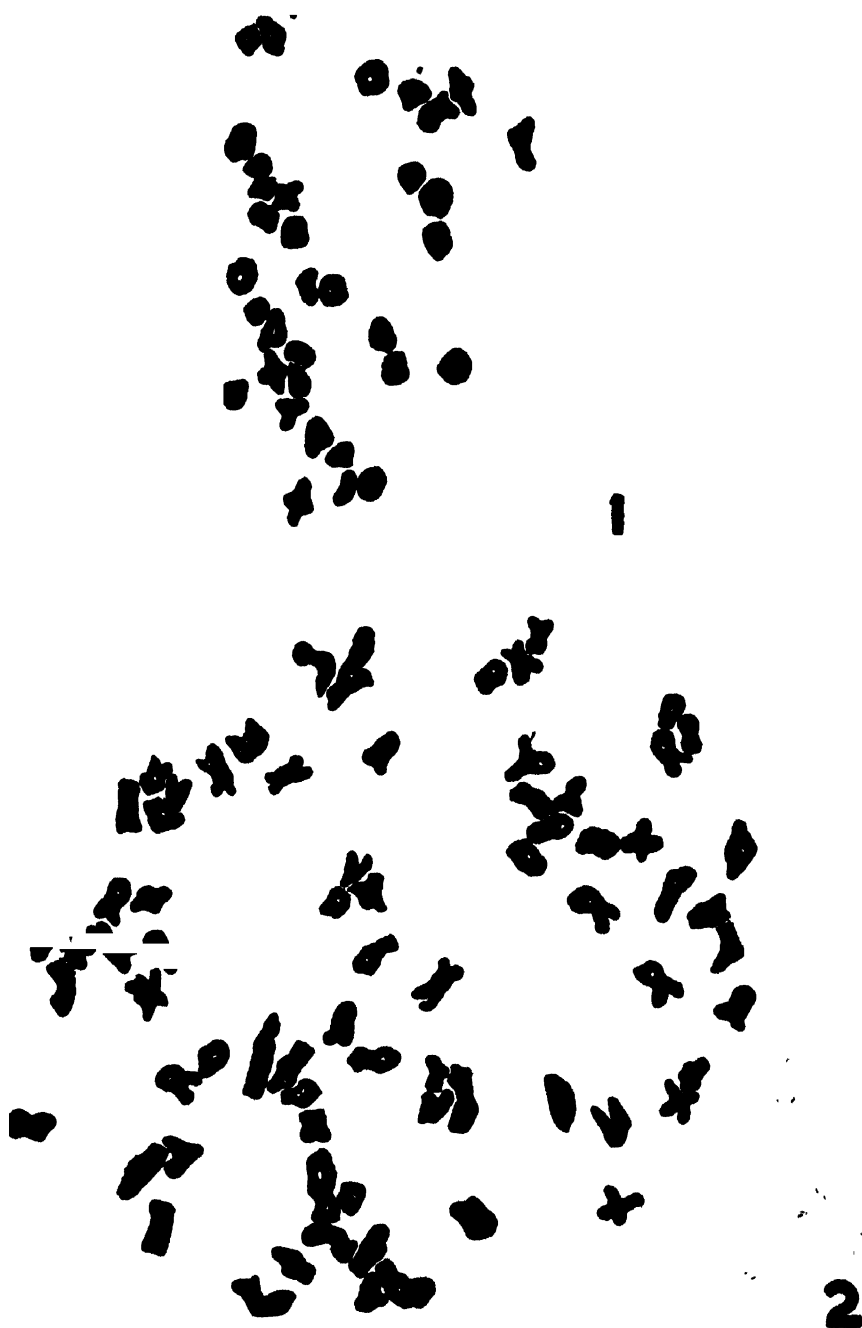
Bower (1928) and Copeland (*loc. cit.*) consider *Stenochlaena* as a Blechnoid member. It has been treated under the Aspidiaceae by Ching (1940) and Dickason (1946). Christensen (1938) placed it with *Acrostichum* in the group of 'Acrostichoid genera derivated from the Pteridoideae'. In this he was followed by Holttum (1949, 1954) who placed it in the sub-family Pteridoideae of his large family Dennstaedtiaceae. Recently Alston (1956) has included it in the family Polypodiaceae (sensu stricto).

The cytological data on the Blechnoid ferns is very meagre. The three genera *Blechnum*, *Woodwardia*, and *Stenochlaena* have collectively about 225 species but to date only a dozen out of these have been worked out. Furthermore, some measure of uncertainty exists about the exact basic chromosome numbers of these genera. The present investigation deals with the meiotic chromosome numbers of the type species of these genera.

*Blechnum orientale* and *Stenochlaena palustris* are essentially low-land ferns growing very abundantly near Teesta, District Darjeeling, between 500–700 ft. altitude. The former is an occupant of rather exposed places along Teesta—Gangtok Road, while the latter is a luxuriant climber in the forest alongside the same road covering tree trunks and reaching tree tops. On the other hand *Woodwardia radicans* has an extensive range, from Kashmir to Bhootan in the Himalayas (Beddome, 1892), occurring on calcareous soil in moist ravines at an elevation of 3,000–8,000 ft.

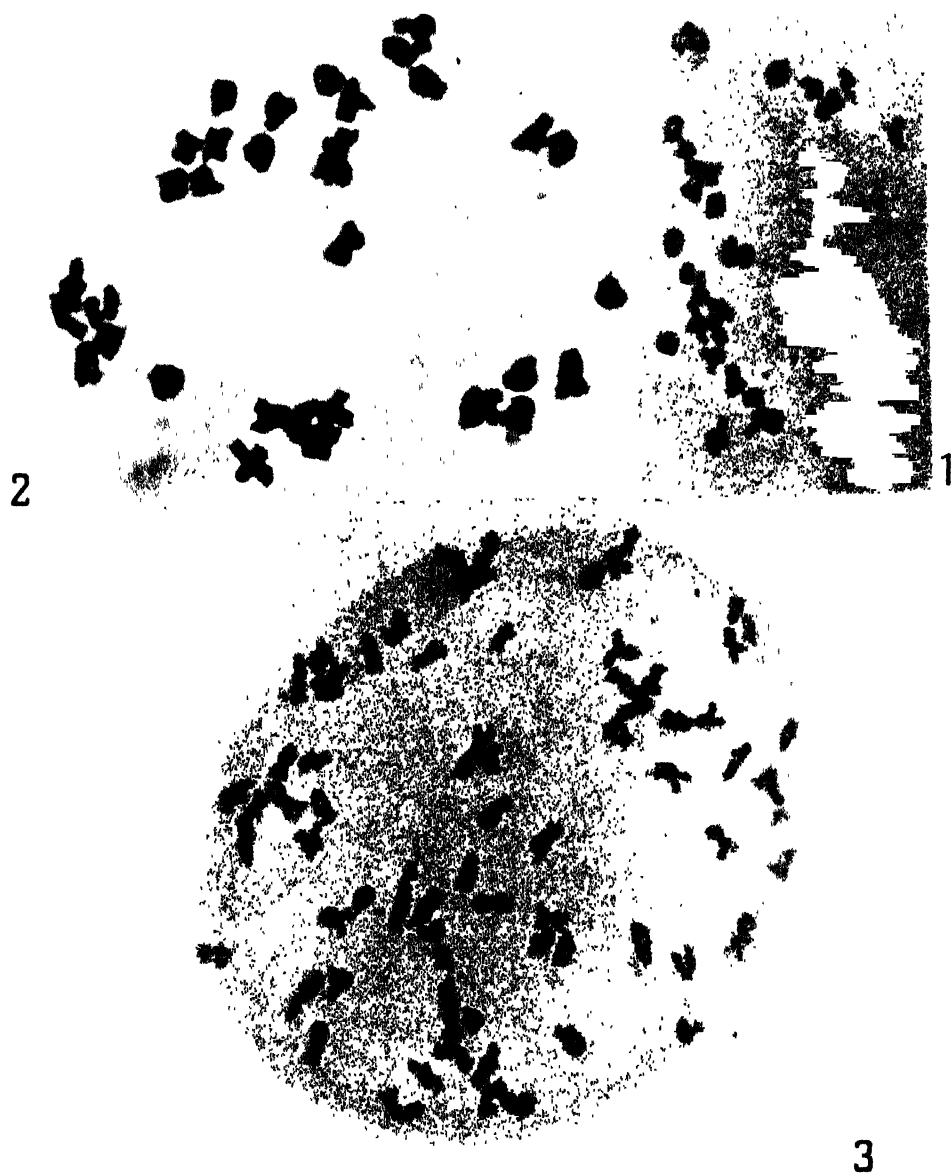
For meiotic chromosome counts the usual aceto-carmin squash technique was employed. The fixations were made in Carnoy's Fluid for 24–48 hrs. Counts were made from a large number of mother cells to ascertain the correct number. In the case of *Woodwardia radicans* the chromosome number was investigated from plants growing in two areas, at Mussoorie in the Western Himalayas, and Darjeeling in the Eastern Himalayas, in both cases with identical results.

In both *Blechnum orientale* and *Woodwardia radicans* the  $n$  number is 34 without doubt (Pl. IV, figs. 1, 2; Text-fig. 1). The meiotic chromosomes in the latter are somewhat larger than in the former and possess a greater affinity for staining. In *Stenochlaena palustris*, on the other hand, 73 bivalents were counted with certainty but these like *Blechnum orientale* take light stain (Pl. IV, fig. 3; Text-fig. 2). The meiosis in all the three species is normal and 64 well filled spores are formed



TEXT-FIG. 1. *Blechnum orientale*. Explanatory diagram to plate IV.  
Fig. 1.  $n = 34$ .  $\times 2100$ .

TEXT-FIG. 2. *Stenochlaena palustris*. Explanatory diagram to plate IV.  
Fig. 3.  $n = 73$ .  $\times 1600$ .



## EXPLANATION TO PLATE IV.

- Fig. 1. A spore mother cell of *Blechnum orientale*,  $n = 34$ .  $\times 1700$ .  
 Fig. 2. A spore mother cell of *Woodwardia radicans* showing  $n = 34$ .  $\times 1300$ .  
 Fig. 3. 73 bivalents at meiosis in a spore mother cell of *Stenochlaena palustris*.  $\times 1300$ .



within a sporangium in each case. The first two plants are diploid while the degree of ploidy in the last one cannot be ascertained until more species belonging to the genus are worked out. But in all probability this is also a diploid with compounded number.

A perusal of the literature shows that different chromosome numbers have been reported for *Blechnum*; *B. spicant* possesses  $n = 34$  and  $2n = 68$  (Manton, 1950) while *B. orientale*, *B. cartilagineum*, *B. procerum* and *B. nudum* are reported to possess  $n = ca\ 33$ ,  $n = 32$ ,  $n = 56$  and  $n = 28$  respectively (Manton and Sledge, 1954). Copeland (*loc. cit.*) considers the genus to be highly diversified and this is fully supported by the different base numbers  $n = 28, 32, 34$  present within the genus.

Britton (1953) reported  $n = 36 \pm 1$  for *Woodwardia* (*Anchistea*, Presl.) *virginica* while Wagner (1955) found  $n = 35$  for the same species. In *Woodwardia* (*Lorinseria*, Presl.) *areolata* Wagner (*loc. cit.*) too reported  $n = 35$ . But in *Woodwardia radicans* and *W. chamissoi* the number is  $n = 34$  (Manton and Sledge, 1954). Copeland (*loc. cit.*) considers *Lorinseria areolata* as a type which he separates from *Woodwardia*. The cytological evidence supports this segregation. Similarly *Anchistea* Presl. typified by *A. virginica* ( $n = 35$ ) which has been merged in *Woodwardia* by Copeland, on the basis of cytology may deserve a generic rank if supported by other characters.

The same chromosome number  $n = 34$  in *Blechnum* and *Woodwardia* (in part at least) confirms a close relationship between the two genera.

Two species of *Stenochlaena* have been investigated by Manton (1954) and Manton and Sledge (1954), in both cases with uncertain chromosome numbers. In *S. palustris*  $n$  is stated to be 70–80 with a probability of 74 and  $2n = ca\ 148$ . The number has now been shown to be  $n = 73$  for this species. In *S. tenuifolia* the report is  $n = 72-74$ . It is obvious that  $n = 73$  for *Stenochlaena* bears no relationship with the numbers reported for the other two afore-mentioned genera.

#### *Affinities of Stenochlaena :*

Sufficient information has now accumulated on the genus *Stenochlaena*, based chiefly on the much investigated species *S. palustris*, to justify assessment of characters for deducing phylogenetic relationship of this controversial genus.

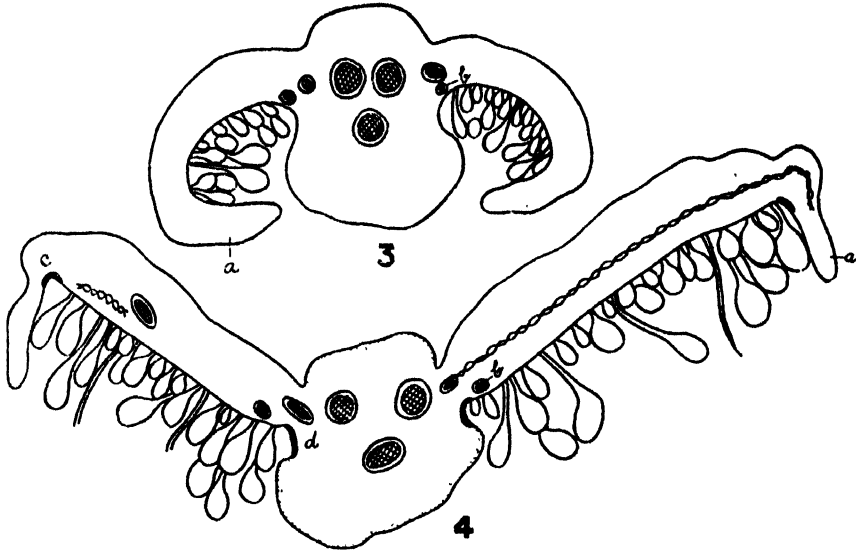
The members of the genus are all tropical climbers which possess usually pinnate dimorphic fronds with stiff texture and parallel venation. The scales borne on the rhizome are ovate-orbicular and unequally peltately attached.

The anatomy of the stem is highly complicated (Mehra and Chopra, 1951). The central vascular system is a dicyostele due to the presence of narrow and much elongated foliar gaps and perforations, both of which open in the same fashion by the departure of a basal root-trace. This main stelar system is enveloped on the outside by a peripheral double system of smaller strands which form reticulate net-works. The peripheral system owes its origin to the basal root-trace strands which on their way out leave a residuum of traces in the cortex before passing out into the roots. The leaf trace, which arises from the central system, is binary (one strand arising from each side of the gap) but is supplemented by several subsidiary strands from the peripheral system before it enters the petiole.

The anatomy of fertile pinna is studied by Holttum (1932). In the rather thick midrib are present three vascular bundles, two upper and one lower. In addition there are present two lateral ones which supply the veinlets to the lamina. Besides these a continuous vein designated as 'soral vein' runs on each side of the midrib at its junction with the lamina and depressed below the general level of veins supplying the lamina. Branches from the latter which supply the sporangia similarly lie at a lower level giving a so-called 'diplodesmic' condition.



The lamina is soriferous all over excepting for some distance from the margins all along its length. A feature of great phyletic interest which has been overlooked by Holttum is that the naked margins of the fertile pinnae are strongly reflexed downwards and inwards not only, in the young state but also in the mature fronds (Text-figs. 3,4) giving the appearance of a 'false indusium'. This is very similar to what has been figured by Bower (1928) in the primitive species of *Blechnum* belonging to the section *Lomaria* which possess a poor development of the 'flange'. The 'incurved indusium' is 4-6 cells in thickness. The junction of the 'indusium' with the lamina is marked on the under-surface by the presence of 2-4 rows of somewhat thick walled, wavy, epidermal cells with brown contents



TEXT-FIGS. 3,4. T. S. of a young and mature fertile pinna, (a) incurved margin (b) soral vein (c) dark cells at the junction of the margin and the lamina (d) similar cells at the junction of the mid-rib with lamina.  $\times 30$

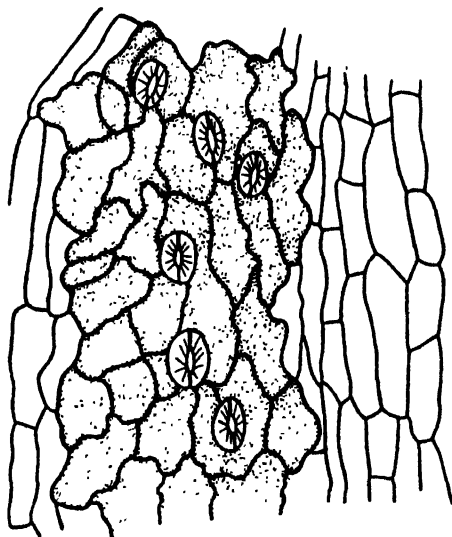
(Text-fig. 5). This region contrasts markedly with the rest of the epidermal surface. Similarly differentiated rows of cells are present at the junction of the midrib with the lamina. Holttum (1932) figured the margins of the fully ripe fertile pinnae reflexed outwards which may probably occur under strong conditions of dessication.

Mixed sporangia of all ages are present in the fertile region. The sporangial stalk is much elongated and three rowed. The spores are wedge-shaped and without a perisporium.

The gametophyte has been elaborately studied by Stokey and Atkinson (1952a). A filament is formed on the germination of spore. Unicellular hairs appear rather early in the history of the gametophyte and are abundantly present at its maturity. The brick-shaped meristem is apical and located within the central notch of the cordate prothallus. The sex organs are similar to those met with in the higher leptosporangiate ferns. The basic chromosome number is  $n = 73$  (present report).

Ching's inclusion of the genus in *Aspidiaceae* is unjustified on several grounds. The spores in *Aspidiaceae* (sensu Ching) invariably possess a perisporium which is lacking in *Stenochlaena*. We have investigated some members of *Aspidiaceae* anatomically and find fundamental differences in the basic plan of the stelar system. There is never a basal root-trace given off in the *Aspidiaceae* before the

opening of a leaf-gap. The root-traces are numerous and these arise at all levels from the meristemes enclosing a leaf-gap. Splitting of the stele through perforations such as we find in *Stenochlaena* is not manifested in Aspidiaceae. The leaf-trace is mostly formed of more than two strands. The venation in Aspidiaceae is also widely different from that in *Stenochlaena*. Furthermore, the characteristic incurving of the margin of the fertile pinnae cannot be accounted for if we were to consider the relationship of *Stenochlaena* with the Aspidiaceae. It is true that in the gametophytic generation the two resemble in the matter of profuse development of unicellular hairs but now these have been shown to be present on gametophytes of ferns of diverse origins (Stokey, 1951). Finally there is no correlation between the chromosome number  $n = 41$  present in most members of the Aspidiaceae and  $n = 73$  observed in *Stenochlaena*.



5

TEXT-FIG. 5. A portion of epidermis from the under face at the region of incurving of margin showing 4 rows of thick walled cells with brown contents.  $\times 210$

Christensen (1938) and Holttum (1954) relate the genus to Pteroids placing it near *Acrostichum*. This also seems unjustified. In *Acrostichum* (as in most other Pteroids) the spores are tetrahedral in strong contrast to the wedge-shaped ones in *Stenochlaena*. Then there is a fundamental difference in the vascular system. In *Acrostichum* the stele is a dictyostele formed in a typical fashion and possesses a few medullary strands in the centre which do not contribute to the leaf-trace (Thomas, 1905). The position is entirely different in *Stenochlaena* where instead of medullary strands, cortical ones are present. The opening of a leaf-gap in *Acrostichum* is never accompanied by the formation of a basal root-trace. Further, the leaf-trace in *Acrostichum* is built on a C-shaped type characteristic of the Pteroids but splits early into numerous ones arranged in the same pattern during its departure from the cauline stele, while it is binary in *Stenochlaena*. In *Acrostichum* characteristic peltate and lobed paraphyses are interspersed among the sporangia but in *Stenochlaena* paraphyses are absent. The gametophytic generation is also widely different in the two cases (Stokey and Atkinson, 1952a,c). As in all the other Pteroids so far investigated, the gametophyte in *Acrostichum* lacks emergences of any kind but in *Stenochlaena* unicellular hairs are present. The meristem in the gametophyte of *Acrostichum* is lateral in strong contrast to its apical position in

*Stenochlaena*. Finally in the strictly Pteroid members starting from the genus *Pteris* in Copeland's Pteridaceae including *Acrostichum*, the  $n$  number is consistently 29 or 30 (Manton and Sledge, 1954), while  $n = 73$  in *Stenochlaena* bears no relationship whatsoever with this number.

Recently Alston (1956) in his paper dealing with the sub-division of the family 'Polypodiaceae' included *Stenochlaena* in the Polypodiaceae (sensu stricto) which calls for some comments. His chief arguments for this suggestion are the wedge-shaped non-perisporiate spores and peltate scales, characters which are in common with the Polypodiaceae. He, however, realises the absence of articulation of the frond with the rhizome in *Stenochlaena* which character is present in the Polypodiaceae. Some members of the Polypodiaceae in which the gametophytes have been investigated, like *Drynaria*, *Pseudodrynaria* (Nayar and Kachroo, 1953; Nayar, 1954) and *Pleopeltis* (unpublished observations of the authors), unicellular hairs are present on the prothalli to a varying degree of the type that are met with in *Stenochlaena* and this may further be assumed to lend support to this relationship. The chief objections to this relationship appear to us to be based on completely different general habit of *Stenochlaena*, stiff texture and parallel venation of its fronds and the marked incurving of the margin of the fertile pinnae. Again, the stelar system in advanced Polypodiaceae is a highly disintegrated schizostele in which the leaf-trace arises in a fashion entirely different from *Stenochlaena*. Several strands of the axis on the side of a leaf depart to form a leaf trace and the opening thus caused is repaired by the adjacent strands moving in. This is fundamentally different from the binary trace of *Stenochlaena* arising from the margins of the leaf-gap. Also the characteristic sclerenchyma strands so commonly present in the Polypodiaceae are absent in *Stenochlaena*.

Bower (1928) considered *Stenochlaena* to be related to the Blechnoid ferns. This suggestion was originally put forward by Smith and has recently been upheld by Copeland (*loc. cit.*) who places it along with *Blechnum*, *Woodwardia*, and a few other genera in the Blechnaceae. This relationship is well supported on the basis of cumulative evidence of characters other than the basic chromosome number which itself is shown to be variable not only within the family but even within members of the same genus. The general habit of the plant, the stiff texture of fronds and parallel venation match very well with species of *Blechnum* excepting that *Stenochlaena* is a climber. But Copeland reports that some species of *Blechnum* have scandent rhizomes. The basic pattern of the main stelar system in *Stenochlaena* is in conformity with what is described by Kachroo (1955a) for *Blechnum* and with the other members of Blechnaceae so far worked out although in no species it is complicated by accessory cortical system of strands. The incurved margin of the fertile pinnae in *Stenochlaena* is reminiscent of a similar situation in primitive member of *Blechnum* (Lomaria section) in which the development of 'flange' is rudimentary. The sporangia possess a three-rowed stalk and the spores are wedge-shaped and without a perisporium in both. The earlier stages of germination of the spore in *Blechnum* and *Stenochlaena* are alike and even the mature gametophytes have a good deal in common, particularly in the profuse development of unicellular hairs (Stokey and Atkinson, 1952a,b). These authors, however, remark that in the two species of *Blechnum*, *B. spicant* and *B. buchtienii* studied by them the antheridia have an elongated basal cell giving a clavate appearance to the antheridium unlike the position in *Stenochlaena palustris* where the basal cell is funnel-shaped and the antheridium globular. This situation described for the above two species of *Blechnum* may not be true for the genus as a whole for in *B. orientale* Kachroo (1955b) subsequently reported the basal cell of the antheridium to be funnel-shaped and the general form of the antheridia globular. The sum total of all these characters leads one to conclude that a real phylogenetic relation-

ship exists between *Stenochlaena* and the members of the family Blechnaceae particularly the genus *Blechnum*.

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# STUDIES ON THE CYTOLOGY AND PHYLOGENY OF THE PTERIDOPHYTES

## II. OBSERVATIONS ON THE GENUS *LYCOPodium*

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### ABSTRACT

1. The cytology of nine species and a few varieties of the genus *Lycopodium* indigenous to South India is described.

2. The different species of the genus investigated in this study are found to possess chromosome numbers like  $n = 48$ ,  $n = 104$ ,  $n = 136$  and  $2n \approx c.405$ . These data taken together with previous observations show that basic numbers like 11, 12, 13 and 17 are characteristic of this genus. In the possession of more than one base number in the different species of a single genus, *Lycopodium* is remarkably different from the other genera of primitive Pteridophytes.

3. It is observed that species with a predominant vegetative mode of reproduction like *L. selago*, *L. lucidulum* (and *Phylloglossum drummondii*) show irregular meiotic behaviour.

4. *L. lucidulum* is shown to be probably of hybrid origin.

5. Cyto-taxonomic evaluation of the various subgenera of the genus *Lycopodium* has shown that the scheme of Walton and Alston is more acceptable. It is suggested that the genus *Lycopodium* may be retained with due recognition of the subgenera, the arrangement of which should be revised, taking into account data of chromosome numbers to supplement other relevant criteria.

### INTRODUCTION

The Lycopods are a great group of primitive microphyllous Pteridophytes, consisting of four living genera, *Lycopodium*, *Phylloglossum*, *Selaginella* and *Isoetes*. Traceable back in geological history to the Carboniferous period in which they reached the climax of their development, the Lycopods were represented by many genera and a great number of species, most of which were characterised by an arboreal habit. Though the living genera differ markedly from their fossil relatives in habit, in the lack of secondary growth and in many other features, the fossils *Lycopodites* and *Selaginellites* from the Upper Carboniferous strata have shown the probability that forms similar in habit to *Lycopodium* and *Selaginella* and perhaps referable to these genera existed even in such a remote period (Eames 1936). The finding of the Devonian *Drepanophycus* and the Silurian *Baragwanathia* (Lang and Cookson 1935) and the discovery of Lycopodiaceous shoots in the Middle Cambrian of East-Siberia (Kryschtofowitch 1953) indicate that the Lycopodiaceous stock can be traced still further back in time.

The living genera can be sharply separated into two groups: (1) eligulate forms with homosporous fructifications and (2) those which are ligulate and heterosporous. *Lycopodium* and *Phylloglossum* are assigned to the former group while *Selaginella* and *Isoetes* come under the latter. The monotypic *Phylloglossum* is confined to Australia and New Zealand, and as no material was available it is excluded from the present study. The cytology of *Isoetes* and *Selaginella* is reported elsewhere (Abraham and Ninan 1958, Ninan 1958a). The present paper deals with the cytology of nine species and a few varieties of *Lycopodium* indigenous to South India.

The genus *Lycopodium*, with nearly 100 species (Eames 1936), possesses very distinctive features and has a cosmopolitan distribution though it occurs in greater abundance in the tropical and subtropical forests. In spite of the world-wide distribution and the great phylogenetic importance of this group, the cytology of only a few species has appeared in previous reports. Difficulty in procuring fresh material at the right stage and the peculiar technical problems associated with their study account for this. Manton (1950) has very rightly pointed out that "*Lycopodium* is the most awkward genus of the Pteridophytes for cytological study".

#### MATERIAL AND METHODS

The materials used in this study were collected from different localities in South India and grown in the Botanical Garden of the Kerala University. Some of the epiphytic species of *Lycopodium* like *L. squarrosum*, *L. macrostachys*, *L. phlegmaria* etc. thrive very well under green house conditions. The terrestrial species like *L. vernicosum*, *L. lucidulum*, *L. wightianum* etc. characteristic of higher elevations do not grow satisfactorily when brought to the plains, though they may survive for a few seasons in the green house.

Fixations for cytological study were made from wild materials wherever possible. In certain cases the transplants also provided material for study. The fixative used was a modified proportion of Carnoy's fluid (Ninan 1955). Actively growing tips of strobili (or shoot apex with young sporangia) were fixed for study of meiosis in spore mother cells. In nearly all cases successful fixations for meiosis were made between 1 p.m. and 3 p.m.

*Lycopodium* is a difficult cytological material; the spore mother cells are thick-walled and it is not easy to break them and get well spaced out chromosome preparations. Secondly, the spore mother cells in most cases are found to contain globule-like bodies making exact cytological interpretation difficult. Added to these, the peculiar chromosome structure in certain species and the tendency for clumping make the *Lycopodiums* the most difficult cytological material among the Pteridophytes. The fixative containing chloroform proved to be very helpful in dissolving out the globule-like bodies and facilitating spreading of the chromosomes when pressure was applied to the cover glass. Sporangia were fixed for nearly a week and a change to fresh fixative was made before smearing. Certain species like *L. lucidulum* combine all these difficulties with irregular pairing and extreme diffuseness of chromosomes, making exact cytological interpretation almost impossible.

#### CYTOLOGICAL OBSERVATIONS

##### *Lycopodium hamiltonii* Spring.

The first material to yield a satisfactory preparation was *L. hamiltonii* Spring. This was collected from Ponmudi (3,500 ft.) and Kodaikanal (7,000 ft.). The Kodaikanal material was epiphytic and pendulous on tree trunks while the Ponmudi material was found to grow on the surface of moist rocks along with mosses and grasses in exposed areas. *L. hamiltonii* Spring. var. *petiolatum* C.B. Clarke (*L. taxifolium* Spring.) was also obtained from Kodaikanal. *L. hamiltonii* is characterised by the lack of specialised cones; the sporangia were distributed in the axils of unaltered leaves in the upper part of the stem. Cytological examination of the Ponmudi materials showed 136 bivalents at first metaphase of meiosis (Pl. V, fig. 1 and Text-fig. 1). The same number of bivalents has also been observed in the Kodaikanal material. The meiotic chromosomes of this species are fairly large compared to those of the other species and have quite normal shape—unlike the "antenna-like" bivalents reported for *L. inundatum* (Manton 1950).



TEXT-FIGS. 1-6

Explanatory diagrams of figures 1-6 on Plate V, reproduced at the same magnification as the photographs, (all  $\times 1000$ ).

Fig. 1. *Lycopodium hamiltonii* Spring.  $n = 136$

Fig. 2. *L. macrostachys* Spring  $n = 136$

Fig. 3. *L. squarrosum* Forst.  $n = 136$

Fig. 4. *L. squarrosum* Forst.  $n = 138$

Fig. 5. *L. wrightianum* Wall ex. Hook & Grev.  $n = 48$

Fig. 6. *L. vernicosum* Hook & Grev.  $n = 136$

*L. vernicosum* Hook. & Grev.

Baker (1887) considers this as a form of *L. hamiltonii* with "much reflexed leaves". They resemble the latter species in external appearance; but are usually of shorter stature with a number of lower leaves almost always drooping down and persistent even after drying up. The stem is densely tufted. This species has been collected from Ponmudi (3,500 ft.). They are seen to occur in plenty at the top of an exposed hillock on moist rocks along with mosses and grasses. Cytologically, this species is similar to *L. hamiltonii*; one hundred and thirty-six bivalents are present in spore mother cells (Pl. V, fig. 6 and Text-fig. 6). The meta-phase chromosomes of this species, unlike those of *L. hamiltonii*, are more fuzzy and exhibit a tendency for clumping. There is no appreciable difference in chromosome size.

*L. squarrosum* Forst. [= *L. ulicifolium* (Vent.) Hook.]

The largest epiphytic species of *Lycopodium* indigenous to this area is *L. squarrosum*. This species occurs in several places in the Western Ghats and was collected from Ponmudi and Munnar areas (2,500–3,000 ft.). This is characterised by the possession of pendulous shoots, one to two feet long and two to three times dichotomously forked. Preparations of meiosis from wild materials of this species collected from Ponmudi (epiphytic on a large rock) showed the presence of 136 bivalents in spore mother cells (Pl. V, fig. 3 and Text-fig. 3). The Munnar material also showed the haploid number of  $n = 136$ . However, materials from one of the earlier collections from Ponmudi (epiphytic on tree trunks) showed 138 bivalents in a spore mother cell (Pl. V, fig. 4 and Text-fig. 4). The meiotic chromosomes of this species are almost comparable to those of *L. hamiltonii* in size and appearance.

*L. macrostachys* Spring. (*L. phyllanthum* Hook. & Arn.)

This is another epiphytic species usually found growing on the trunks of huge trees at higher elevations. They form very big clumps and are characterised by luxuriant vegetative growth. One such clump was recently collected from Ponmudi. The enormous size of this can be well appreciated from the fact that it had over 500 shoots. In this species, the shoots are more fleshy and stout and the leaves are moderately dense, spreading, with revolute edges and distinct midrib, and larger than those of any other species collected so far. The strobili are highly differentiated from the vegetative axis and are dichotomously branched a few times. In certain materials of this species, the growth of the strobili is seen to continue and give rise to vegetative shoots. This species occurs in several places in the Western Ghats namely, Ponmudi (3,500 ft.), Poringalkuthu (2,500 ft.), Thekkady (2,000 ft.) etc. Cytological examination of the Ponmudi material showed 136 bivalents in spore mother cells (Pl. VII, fig. 11 and Text-fig. 11). The bivalents are of quite normal shapes and closely resemble those of *L. squarrosum*. The same number of bivalents has also been observed in spore mother cells of all the other materials of this species collected from different localities.

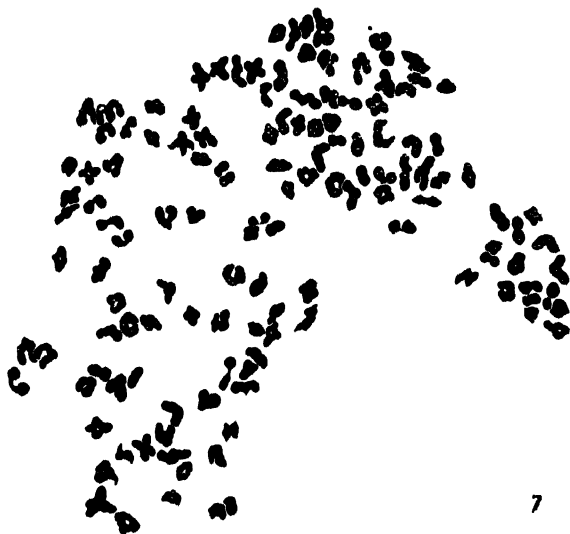
Another variety of *L. macrostachys* obtained from Kodaikanal hills (7,000 ft.), showed much thicker strobili, reaching almost a foot in length. Cytological examination of this also showed the chromosome number to be exactly  $n = 136$  (Pl. V, fig. 2 and Text-fig. 2). The bivalents here again are of normal shapes and almost comparable to those of the Ponmudi material.

*L. phlegmaria* L.

This is another epiphytic species occurring in similar habitats as *L. macrostachys* and almost resembling it in external appearance. Unlike the former, the vegeta-



tive axis in *L. phlegmaria* is less stout and pendulous. The strobili which are highly differentiated from the vegetative shoots are profusely branched in a dichotomous manner and are very slender. In external appearance, this closely resembles *L. nummularifolium*. *L. phlegmaria* grows in several places in the Western Ghats, like Ponnudi, Munnar, Thekkady and Poringalkuthu. This is the only species of *Lycopodium* so far found in coastal areas. It was collected from Sherthalai



TEXT-FIGS. 7-8

Explanatory diagrams to figures 7-8 on Plate VI, reproduced at the same magnification as the photographs. (both figures  $\times 1000$ ).

Fig. 7. *L. phlegmaria* L.  
Fig. 8. *L. lucidulum* Michx.

$n = 136$   
 $2n = c.405$ .

(sea-coast), from a *Calophyllum inophyllum* tree on which it was growing epiphytically. Epiphytic races of this are also seen to occur in the plains. Chromosome counts of  $n = 136$  were made from materials collected from all the above localities. The meiotic chromosomes in the Sherthalai material of this species are illustrated in Pl. VI, fig. 7 (see also Text-fig. 7).

*L. setaceum* Hamilt.

This epiphytic species was collected from Ponmudi, Poringalkuthu and Muthukuzhivayal in the Western Ghats. The Muthukuzhivayal material was considerably different from the other two in external appearance. However, cytological examination of all the three gave identical results. One hundred and thirty-six bivalents were counted from spore mother cells. The illustration (Pl. VII, fig. 13) shows the meiotic chromosomes of this species collected from Poringalkuthu. Meiosis in the Muthukuzhivayal material is illustrated in Text-figure 14. The meiotic chromosomes of this species are slightly smaller than those of all the other species with  $n = 136$ .

*L. cernuum* L.

This terrestrial species is very common on way sides at higher elevations. It is also seen to occur in certain areas in the plains. The vegetative shoots are highly branched, each branch at maturity ending in a compact cone. The branching in this species is apparently monopodial. Chromosome counts from materials of this species collected from Trivandrum showed 104 bivalents at metaphase of meiosis (Pl. VII, fig. 12 and Text-fig. 12). The bivalents in this species are seen to be much smaller than those of all the other species investigated in this study.

*L. wightianum* Wall. ex Hook. & Grev.

This is typically a terrestrial species occurring in abundance around the Fairy Falls, Kodaikanal hills. This is characterised by the possession of wide-trailing stems with many ascending branches and branchlets. Cytological examination of materials of this species fixed at Kodaikanal and brought to Trivandrum showed the presence of 48 bivalents in spore mother cells (Pl. V, fig. 5 and Text-fig. 5). The bivalents at diakinesis exhibit very peculiar shapes and are of different sizes, presenting difficulties in interpretation. Hence it is very difficult to get clear chromosome counts even though the number of bivalents is relatively low. This species exhibits the lowest number of chromosomes in any species of *Lycopodium* investigated in the present study.

*L. lucidulum* Michx. (= *L. reflexum* Sw.)

This terrestrial species was obtained from Bear Shola, Kodaikanal hills. It grows abundantly on the sides of a stream in a shady place. The plant is small and the stem suberect and one to three times dichotomously forked. The stem is covered by a large number of closely arranged leaves which diverge away in a drooping manner. This species is further characterised by the presence in the axils of the upper leaves, of "bulbils" which drop to the ground and develop into new plants. From careful examination made in several seasons it appears that vegetative propagation is well established in this species.

The cytological difficulties inherent in the *Lycopodiums* reach the climax in *L. lucidulum*. The meiotic chromosomes in this species are very fuzzy structures with diffuse outlines and the pairing is very irregular. Some of the bivalents (or multivalents, if they are present) can hardly be distinguished from some of the univalents. This makes quantitative enumeration of chromosome number very

difficult. Chromosome counts even from very clear and well spaced out preparations can therefore be only approximate. As Manton (1950) has suggested, the actual number is not as significant as the presence of univalents. One hundred and eighty bivalents and 45 univalents were counted from a clear preparation of a spore mother cell (Pl. VI, fig. 8 and Text-fig. 8). The sporangia used in the study



TEXT-FIGURES 11-14

Fig. 11. Explanatory diagram to fig. 11 on Plate VII, showing 136 bivalents in *L. macrostachys* Spring. (variety with short strobili).  $\times 1000$ .

Fig. 12. Explanatory diagram to Plate VII; fig. 12  $n = 104$ .  $\times 1000$ .

Fig. 13. Explanatory diagram to Plate VII, fig. 13 showing  $n = 136$  in *L. setaceum* Hamilt.  $\times 1000$ .

Fig. 14. Diagram of meiosis in *L. setaceum* Hamilt. (variety from Muthukuzhivayal).  $n = 136$ .  $\times 1000$ .

of meiosis were fixed in the field and preparations of meiosis were made in two consecutive years. Irregular pairing was seen to be a constant feature in all the preparations of meiosis and it is believed from this, that the failure of pairing is not due to metabolic causes (as is seen in certain garden materials in the first season of transplanting), but to lack of homology among the chromosomes. This was further confirmed from spore mother cells showing bivalents and lagging univalents at first metaphase of meiosis (Pl. VII, fig. 9). Laggards were also seen at first anaphase of meiosis (Pl. VII, fig. 10). The irregular pairing therefore is in all probability an indication of hybridity. If it is really so, it would suggest comparison to the situation encountered in *L. selago*, described by Manton (1950) as "the most ancient impure species cytology has so far detected". Failure of chromosome pairing at meiosis has also been demonstrated in the related species *Phylloglossum drummondii* Kunze (Blackwood 1953). On comparison of these three species it is seen that vegetative propagation through "bulbils" is characteristic of *L. selago* and *L. lucidulum* while *Phylloglossum* propagates through "tubers". The prevalence of accessory methods of propagation in these species may be related in some way with the abnormalities in the meiotic process—the disadvantages arising from a defective meiosis might be counter-balanced by the parallel attainment of a vegetative mode of reproduction.

Though other species like *L. serratum*, *L. clavatum*, *L. nummularifolium*, *L. subulifolium*, *L. casuarinoides* etc. were obtained, they could not be studied due to lack of sporangial material at the proper stage of development. It may be possible to report on them on a later occasion.

## DISCUSSION

### *Phylogenetic considerations :*

On comparison of the observed chromosome numbers with previous reports on the cytology of this genus, the first and the most striking thing that one gathers is the remarkable variation of chromosome numbers exhibited by the different species of this genus. The list of chromosome numbers of the species so far investigated is given in the table below.

TABLE I  
*Chromosome numbers in Lycopodium*  
(Present study)

| species                                     | source   | chromosome number                     |
|---|----------|---------------------------------------|
| <i>L. lucidulum</i> Michx.                  | S. India | irregular meiosis<br>( $2n = c.405$ ) |
| <i>L. hamiltonii</i> Spring                 | "        | $n = 136$                             |
| <i>L. vernicosum</i> Hook & Grev.           | "        | $n = 136$                             |
| <i>L. squarrosum</i> Forst.                 | "        | $n = 136$                             |
| <i>L. macrostachys</i> Spring               | "        | $n = 136$                             |
| <i>L. phlegmaria</i> L.                     | "        | $n = 136$                             |
| <i>L. setaceum</i> Hamilt.                  | "        | $n = 136$                             |
| <i>L. cernuum</i> L.                        | "        | $n = 104$                             |
| <i>L. wightianum</i> Wall. ex Hook. & Grev. | "        | $n = 48$                              |

TABLE I (contd.)  
(Previous reports)

| species                    | source        | chromosome number |           | author                    |
|----------------------------|---------------|-------------------|-----------|---------------------------|
|                            |               | <i>n</i>          | <i>2n</i> |                           |
| <i>L. inundatum</i> L.     | Scotland      | 78                | —         | Manton 1950               |
| <i>L. annotinum</i> L.     | Sweden        | 34                | —         | "                         |
| <i>L. annotinum</i> L.     | Lake District | —                 | c. 68     | "                         |
| <i>L. clavatum</i> L.      | "             | 34                | 68        | "                         |
| <i>L. selago</i> L.        | "             | irregular         | c. 260    | "                         |
| <i>L. alpinum</i> L.       | Wales         | 24-25             | c. 48     | "                         |
| <i>L. clavatum</i> L.      | N. America    | —                 | c. 60     | Dunlop 1949               |
| <i>L. complanatum</i>      | "             | —                 | 40        | "                         |
| <i>L. annotinum</i> L.     | "             | —                 | c. 50     | "                         |
| <i>L. obscurum</i>         | "             | —                 | c. 50     | "                         |
| <i>L. complanatum</i>      | —             | 22                | —         | Harmsen (cf. Delay 1953). |
| <i>L. clavatum</i> L.      | —             | 14                | —         | Baranov 1925              |
| <i>L. clavatum</i> L.      | N. India      | 34                | —         | Mehra and Verma 1957      |
| <i>L. nikoense</i>         | Japan         | 34                | —         | "                         |
| <i>L. lucidulum</i> Mich.  | N. India      | 132               | —         | "                         |
| <i>L. setaceum</i> Hamilt. | "             | 165-170           | —         | "                         |

(In scrutinising the above list it is at once obvious that some of the earlier observations are incorrect and based on erroneous interpretations. Manton (1950) alone of all the authors has given credible photographic evidence of chromosome preparations. Others have provided only diagrams of chromosomes, which by itself is no convincing evidence, especially in view of the fact that they are conflicting observations. In the present study the author has adopted the principle : "what cannot be photographed cannot be used as evidence" (Manton 1950). For purposes of discussion therefore only those data which are well authenticated alone will be referred to.)

The haploid numbers  $n = 78$  and  $n = 104$  in *L. inundatum* (Manton 1950) and *L. cernuum* (present study) respectively are traceable back to a basic number of 13. It is already shown from evidences of chromosome numbers in ancient genera like *Psilotum*, *Marattia*, *Matonia*, *Hymenophyllum*, *Dicranopteris* etc. that chromosome number 13 might have been widely prevalent in the past in primitive groups of Pteridophytes (Manton 1950, 1954 ; Manton and Sledge 1954 ; Ninan 1956b,c). It is very interesting to note that the primitive genus *Lycopodium* also exhibits in some of its species numbers which are referable to 13 chromosomed ancestral types. Haploid numbers like  $n = 34$  in *L. clavatum* (Manton 1950, Mehra and Verma 1957), *L. annotinum* (Manton 1950) and *L. nikoense* (Mehra and Verma 1957) and  $n = 136$  in South Indian species like *L. hamiltonii*, *L. macrostachys*, *L. vernicosum*, *L. setaceum*, *L. phlegmaria* and *L. squarrosus* observed in the present study clearly show that they might have had a common cytological origin, presumably from 17 chromosomed ancestral types. *L. alpinum* (Manton 1950) and *L. wightianum* (present study) with haploid numbers like  $n = 24-25$  ( $2n = c.48$ ) and  $n = 48$  respectively represent another distinct evolutionary line, probably from ancestral types with 12 as the basic chromosome number. Harmsen's report (Delay 1953) of  $2n = 22$  in *L. complanatum* and the purported presence of chromosome numbers in multiples of 11 in a few species of *Lycopodium* (Mehra and Verma 1957) show that the basic number 11 is characteristic of some species of this genus. Other primitive genera like *Isoetes* (Abraham and Ninan 1958) and *Osmunda* (Manton 1950, 1954 ; Ninan 1956d) also show the base number 11. It is thus seen that all the investigated species of the genus *Lycopodium* (with authentic chromosome counts) are referable to basic numbers like 11, 12, 13 and 17. In

this respect the genus *Lycopodium* provides a striking contrast to the situation in other genera of Pteridophytes, most of which show in the different species of a genus, numbers which are exact multiples of a base number characteristic of that genus. Haploid chromosome numbers like  $n = 120, 240, 480$  etc. in species of *Ophioglossum* (Ninan 1956a, 1958b) and those like  $n = 11, 22, 33, c.55$  etc. in *Isoetes* (Manton 1950, Abraham and Ninan 1958) are examples to this. In the leptosporangiate ferns also, the same relationship between the various species of a genus is found to hold good. However *Lycopodium* shows a different cytological situation in possessing different basic numbers in different species. But fossil history tells us that the Lycopodiaceous stock is referable back to the Siluro-Devonian strata, and the surviving species need be regarded only as representing end members of distinct phyletic lines, though in view of the stereotyped morphological features and close similarity in technical characters, some of them have come to be considered as constituting different species of a single genus. As Manton (1950) has remarked "the cytological evidence as a whole can only underline their complete dissimilarity from one another, and one must recognize in them representatives of phyletic lines which have been so long separated that their cytological connexion, if it ever existed, has become completely obscured. They seem now to be far more different from each other than are the genera or even groups of genera of the Polypodiaceous ferns. This is perhaps a sign of antiquity".

#### *Cyto-taxonomy of the genus Lycopodium :*

All the species of the Lycopodiaceae, with the exception of the monotypic *Phylloglossum*, are united into the single genus *Lycopodium*. Goebel (1930) divides this comprehensive genus into five groups, taking into account the characters of the gametophyte. The five groups according to Goebel are *Selago*, *Phlegmaria*, *Cernuum*, *Clavatum* and *Complanatum*. Pfitzer recognizes two subgenera based on the character and arrangement of the sporophyll, namely, *Urostachya* (including two sections, *Selago* and *Phlegmaria*) and *Rhopalostachya* (with three sections, *Inundata*, *Cernuua* and *Clavata*). Baker's (1887) scheme of classification, again based on character and arrangement of sporophylls, recognizes four subgenera, *Selago*, *Subselago*, *Lepidotis* and *Diphasium*, each of which is again subdivided into different sections. Walton and Alston (1938) proposed another scheme following that of Herter in the main. This recognizes six subgenera, *Urostachys*, *Clavato-stachys*, *Complanatostachys*, *Cernuostachys*, *Inundatostachys* and *Lateralistachys*.

The above systems of classifications, with the exception of that of Goebel, are based on the character and arrangement of sporophylls. It is clearly evident that any classification which does not take into account data for comparison from as many criteria as possible cannot be natural. Taking Baker's scheme, for instance, it is seen that there are certain obvious discords in it in the light of evidence from cytology. The table provided below would serve to illustrate this.

It is clear from the table that Baker's subgenus *Lepidotis* is a heterogeneous assemblage of species with haploid numbers like  $n = 136, 104, 78, 34$  and  $24-25$  which in turn are traceable to base numbers like 17, 13 and 12. In the *Clavatum* section of *Lepidotis* itself are included species like *L. clavatum* and *L. annotinum* with  $n = 34$  and *L. alpinum* with  $n = 24-25$ . In a natural arrangement of these species, it is essential that if morphological evidences would provide sufficient warranty, species which are evidently related in a cytological sense should be grouped together. In that case, it is necessary to remove the *Phlegmaria* section of Baker's subgenus *Lepidotis* to the subgenus *Selago* to which may be merged the next subgenus *Subselago*. The single large group which would thus result would be traceable to a basic number of 17. Turning now to the scheme of Walton and Alston (1938), it is seen that exactly these three sections (*Selago*, *Subselago*

TABLE II

*Chromosome numbers in Lycopodium species*

(arranged according to Baker's system)

| section                     | species                | chromosome number | basic number |
|-----------------------------|------------------------|-------------------|--------------|
| Subgenus : <i>Selago</i>    |                        |                   |              |
| <i>Selago</i>               | * <i>L. selago</i>     | $2n = c.260$      | 17?          |
|                             | <i>L. lucidulum</i>    | $2n = c.405$      | 17?          |
|                             | <i>L. hamiltonii</i>   | $n = 136$         | 17           |
| <i>Taxifolium</i>           | <i>L. setaceum</i>     | $n = 136$         | 17           |
| Subgenus : <i>Subselago</i> |                        |                   |              |
|                             | <i>L. squarrosum</i>   | $n = 136$         | 17           |
| Subgenus : <i>Lepidotis</i> |                        |                   |              |
| <i>Inundatum</i>            | * <i>L. inundatum</i>  | $n = 78$          | 13           |
| <i>Phlegmaria</i>           | <i>L. phlegmaria</i>   | $n = 136$         | 17           |
|                             | <i>L. macrostachys</i> | $n = 136$         | 17           |
| <i>Cernuum</i>              | <i>L. cernuum</i>      | $n = 104$         | 13           |
| <i>Clavatum</i>             | * <i>L. clavatum</i>   | $n = 34$          | 17           |
|                             | * <i>L. annotinum</i>  | $n = 34$          | 17           |
|                             | * <i>L. alpinum</i>    | $n = 24-25$       | 12           |
| Subgenus : <i>Diphasium</i> |                        |                   |              |
|                             | <i>L. wightianum</i>   | $n = 48$          | 12           |

\*Reports of Manton (1950). Other determinations are made in the present study.

and *Phlegmaria*) are united by them to form the subgenus *Urostachys*. Species with a basic number of 17 in the *Clavatum* section (*L. clavatum* and *L. annotinum*) are evidently related to the *Phlegmaria* section in a cytological sense. However, Walton and Alston regard the *Clavatum* group of species as a distinct subgenus, the *Clavatosachys*. Both the *Urostachys* and the *Clavatosachys* of Walton and Alston are thus seen to have the same basic chromosome number. However, in view of the morphological differences exhibited by the two groups, Walton and Alston's treatment of them as two distinct subgenera seems quite justified. The morphological distinctness of the *Inundatum* and *Cernuum* sections has been stressed by all the authors. Walton and Alston raise them to the status of subgenera. The present study of representative species of these two subgenera has shown that despite morphological differences they are cytologically related in the possession of a common basic number 13. To make further discussions on the scheme of Walton and Alston, knowledge of chromosome numbers from representative species of the other subgenera is necessary. However, as far as is known, the scheme of Walton and Alston seems to be the most satisfactory. Further knowledge of chromosome numbers of different species is likely to be helpful in arriving at more correct taxonomic grouping of this complicated genus.

The question now remains whether or not the various subgenera should be raised to generic rank. Campbell (1939) remarks : "the differences shown by the

gametophyte and sporophyte are so great that it does not seem logical to refer all the species to a single genus." Herter raises the subgenus *Urostachys* to generic rank (Walton and Alston 1938). As far as this genus is concerned, the erection of new genera out of one or more of the subgenera is not warranted in the light of cytological evidence, since the same basic number is seen to be repeated in more than one section. The genus *Lycopodium* may be retained as such with due recognition of the various subgenera, the arrangement of which should be revised on the lines indicated above.

It is generally conceded that forms without definite cones are the more primitive and that specialisation into definite strobili is an advanced character. Bower (1935) holds that vegetative leaves are sterilised sporophylls and that the most primitive types are those in which almost every leaf bears a sporangium. *L. compactum*, *L. trencilla*, *L. lucidulum*, *L. selago* etc. would accordingly represent the most primitive types, while *L. wightianum*, *L. cernuum* etc. have to be taken as relatively advanced. Cytological study shows that species like *L. selago*, *L. lucidulum* etc. have comparatively high chromosome numbers ( $2n = 260$  in *L. selago* and  $2n = c.405$  in *L. lucidulum*) and while this may be taken as an indication of relative antiquity of the species concerned, it is by no means a primitive chromosome situation.

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## EXPLANATION OF PLATES

### PLATE V

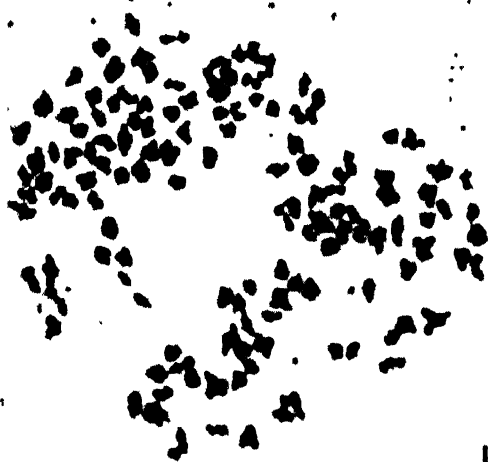
- Fig. 1. First meiotic metaphase in a spore mother cell of *Lycopodium hamiltonii* Spring. from Ponmudi showing 136 bivalents.  $\times 1000$ .
- Fig. 2. A spore mother cell from the Kodaikanal material of *L. macrostachys* Spring. (with long strobili). The number of bivalents is 136.  $\times 1000$ .
- Fig. 3. Meiotic metaphase in *L. squarrosum* Forst. from Ponmudi showing 136 bivalents in a spore mother cell.  $\times 1000$ .
- Fig. 4. Meiosis in *L. squarrosum* Forst. from Ponmudi area showing 138 bivalents in a spore mother cell.  $\times 1000$ .
- Fig. 5. Meiotic metaphase in a spore mother cell of *L. wightianum* Wall. ex Hook. & Grev. from Kodaikanal.  $n = 48 \times 1000$ .
- Fig. 6. A spore mother cell from the Ponmudi material of *L. vernicosum* Hook. & Grev., showing 136 bivalents at metaphase of meiosis.  $\times 1000$ .

### PLATE VI

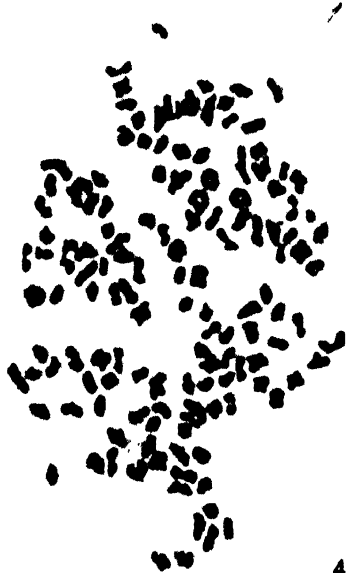
- Fig. 7. Meiosis in *L. phlegmaria* L. from Sherthalai (sea-coast).  $n = 136$ .  $\times 1000$ .
- Fig. 8. Diakinesis in *L. lucidulum* Michx. 180 bivalents and 45 univalents are present (for explanatory diagram see Text-fig. 8).  $\times 1000$ .

### PLATE VII

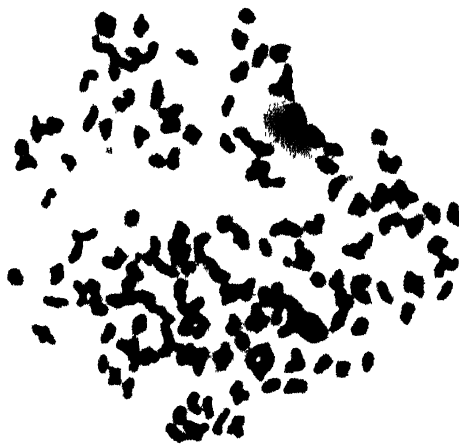
- Fig. 9. A side view of the metaphase plate in a spore mother cell of *L. lucidulum* Michx. showing bivalents and univalents.  $\times 1000$ .
- Fig. 10. Anaphase in a spore mother cell of *L. lucidulum* Michx. showing irregular separation and lagging of chromosomes.  $\times 1000$ .
- Fig. 11. First meiotic metaphase in *L. macrostachys* Spring. (with short strobili).  $n = 136$ .  $\times 1000$ .
- Fig. 12. Meiotic metaphase in a spore mother cell of *L. cernuum* L. 104 bivalents are present.  $\times 1000$ .
- Fig. 13. Metaphase I in a spore mother cell of *L. setaceum* Hamilt. showing 136 bivalents.  $\times 1000$ .



1



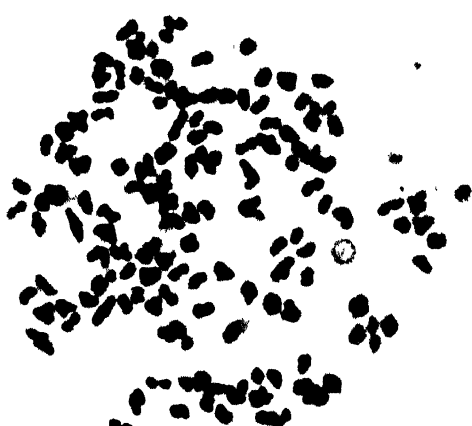
4



2



5



3



6



7



8



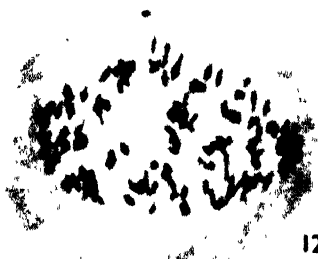
9



10



11



12



13



# A NEW APPROACH TO THE STUDY OF GROWTH-GRADIENT IN THE SEGMENTS OF THE SECOND PAIR OF CHELIPEDS OF THE INDIAN FRESHWATER PRAWNS, *PALAEEMON HENDERSONI* DeMAN (CRUSTACEA : DECAPODA PALAEMONIDAE)

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(Communicated by W. D. West, F.N.I.)

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## ABSTRACT

The author of the present paper has mentioned (Misra, 1957) that the simple allometry formula should be regarded as only a valuable first approximation of the more general formula on differential growth viz.

$$y = bx^{(a+ax)} e^{cx},$$

where  $b$ ,  $a$ ,  $a$  and  $c$  are constants.

In the present paper study of growth-gradient within the five segments, Is., Me., Ca., Pro. and Dac., of the second cheliped of a sample of 90 male and 69 female freshwater prawns of *Palaemon hendersoni* DeMan has been done in the light of this new formula in which it has been shown by the author (*op. cit.*) that the equilibrium constant  $a$  of the simple allometry formula should be replaced by  $\rho$  where,

$$\rho = a + (a+c)x + ax \log_e x.$$

The following points, in particular, have been noted :—

- (i) As anticipated by Huxley (1932), the values of the equilibrium constant  $a$  for the cheliped or its segments are, within the limits of error probably due to the fluctuations of sampling and insufficient size of the data, only the means of the values of corresponding  $\rho$  calculated for different values  $x$  of the carapace length.
- (ii) It is seen that there is a progressive change in the successive values of the growth-coefficient. Thus, the first phase of growth in the male cheliped starts with a high point in Ca., which shifts towards the end of the phase to Me. In the female it shifts from Pro. to Me. The second phase of growth in the male cheliped starts with only one high point (at Pro.) and later another high point, though less steep than the first, develops in Me.
- (iii) The growth-gradient graph, as exhibited by the  $a$  values (which has so far been the method commonly followed) does not indicate the final order of values of the growth-coefficient for the segments of the cheliped. On the other hand, as the  $a$  values are identical with the values of mean  $\rho$ , it can be said to show a pattern obtained by pooling together of the patterns in the various stages of growth during a phase.
- (iv) The values of  $\frac{d\rho}{dx}$ , associated with the values of  $\rho$ , give proper assessment of the significance of the shift and of the existence of the growth-centre within the cheliped.
- (v) It is interesting to note from the present study that the growth pattern in the female cheliped need not be identical with the one in the male (I Phase). In this connection certain points have been discussed in the present paper to compare the growth behaviour of the male cheliped with that of the female.

## INTRODUCTION

Early this century it has been shown by D'Arcy Thompson (1942) and others that with the increase in the body size there takes place a gradual change in the relative sizes of parts in all organic forms, except some of the simplest ones. The

fact, however, that an organic form is the result of the differential growth was successfully established by Julian S. Huxley (1932) who showed by actual quantitative analysis that the relationship,

$$y = bx^k \text{ (now written as } y = bx^a),$$

called the simple allometry formula or equation, between the body size  $x$  and the organ size  $y$  occurs in most of the animals and plants. Huxley and others have demonstrated the immense importance of the simple allometry formula in studying various cases such as those in which the change of proportions in parts of an adult form takes place either with the progressive increase in the body size in species of animals which are related with one another or in an evolutionary course.

Although there has been severe criticism about the applicability of the allometry formula in theory as well as in practice, its importance has undoubtedly to be recognised in certain matters such as the opening of an entirely new approach that the formula has been able to make in explaining the occurrence of growth-gradient patterns in a limb whose rate of growth is different from that of the body. It can be said that it has increased the value of (although very novel but incomplete owing to absence of a definite mathematical expression) the method of Cartesian transformation as suggested by D'Arcy Thompson (*op. cit.*), to explain the inherent commonness in shapes of related forms which apparently look like different species.

Some of the accepted shortcomings of the allometry formula have been pointed out by Huxley (*op. cit.*) himself. Thus, the constant value of  $\alpha$  used in allometry is unable to explain the gradual change in the growth activity that must be taking place within and between parts of region under study, which can be explained properly only by a progressively changing value of the growth-coefficient from point to point along the axis of an organ.

The author of the present paper has already mentioned (Misra, 1957) that the simple allometry formula should be regarded as only a first approximation to the general formula of differential growth, for which the following expression has been derived :

$$y = bx^{(\alpha+ax)}e^{cx}, \quad \dots (1)$$

where  $b$ ,  $\alpha$ ,  $a$  and  $c$  are constants.

In the present paper study of the growth-gradient in growth intensity of the lengths of the joints Ischium, Merus, Carpus, Propodus and Dactylus (for which the abbreviations Is., Me., Ca., Pro. and Dac. will be used henceforth) has been made in the light of this new formula, in which it has been shown by the author (*op. cit.*) that the equilibrium constant  $\alpha$  of the simple allometry formula should be replaced by  $\rho$  where,

$$\rho = \alpha + (a+c)x + ax \log_e x \quad \dots (2)$$

In crustacean chelipeds there are seven such joints, but out of these the first two, viz. coxa and basis, are firmly fused with Is. and are of insignificant length when compared to the total size of the cheliped. Therefore, in the present discussion only five joints, mentioned above, are taken for study.

Decapoda crustacea provides interesting material for the study of relative growth and it will not be out of place to mention here that the concept of growth-gradient, growth-potential etc. has been mostly based on researches conducted on crustaceans of this group. The genus *Palaemon*, which is very common in tropical and sub-tropical freshwater, is a Decapod crustacea belonging to the group

**Macrura.** An interesting feature of the species under present discussion, like many other species of *Palaemon*, is the enormous size that the second pair of chelipeds attains in males after a certain age.

### MATERIAL AND MEASUREMENTS

The material, consisting of 90 male and 69 female prawns of *Palaemon hendersoni* DeMan, was kindly lent to the author by the Director, Zoological Survey of India, and all the specimens on which the present work is based are deposited in the reserve collections of the Zoological Survey.

The lengths are in millimetres, and were taken with sliding calipers fitted with graduated dial reading directly upto the first place of decimal, in the following manner :—

- (i) Length of carapace was measured in a straight line between the orbital edge and the posterior border.
- (ii) Lengths of the segments of the cheliped were measured dorsally in a single straight line.
- (iii) The total length of the cheliped was calculated by adding up the lengths of the individual segments.

Also, only the larger of the second cheliped (which is sometimes dextral and sometimes sinistral) was measured, for basically the chelipeds of the two sides should grow at the same rate and the apparent asymmetry that is sometimes noticed in this species appears to be due to some extraneous factors like autotomy and regeneration.

### OBSERVATIONS AND DISCUSSION

In females the 69 values of log carapace length, denoted by  $X$ , and of log length of the cheliped or its joint, denoted by  $Y$  (the base of logarithm being 10), were condensed to 12 groups with equal intervals for  $X$ . Table I gives the values of group averages for  $X$  and  $Y$ . A similar table for the male chelipeds has been given earlier by the author (*op. cit.*).

TABLE I

*The values of the group averages  $X$  and  $Y$  in females*

| Group     | Average $X$ | Average $Y$ |        |        |        |        |          |
|-----------|-------------|-------------|--------|--------|--------|--------|----------|
|           |             | Is.         | Mo.    | Ca.    | Pro.   | Dac.   | Cheliped |
| 0.55—0.60 | 0.5911      | 0.3010      | 0.2553 | 0.2304 | 0.3010 | 0.3617 | 0.9912   |
| 0.60—0.65 | 0.6368      | 0.3212      | 0.2783 | 0.2382 | 0.3081 | 0.3899 | 1.0086   |
| 0.65—0.70 | 0.6751      | 0.3674      | 0.3148 | 0.2784 | 0.3489 | 0.4089 | 1.0445   |
| 0.70—0.75 | 0.7376      | 0.4093      | 0.3552 | 0.3151 | 0.3799 | 0.4259 | 1.0779   |
| 0.75—0.80 | 0.7761      | 0.4353      | 0.3753 | 0.3516 | 0.4148 | 0.4510 | 1.1062   |
| 0.80—0.85 | 0.8387      | 0.4939      | 0.4346 | 0.3872 | 0.4870 | 0.5330 | 1.1688   |
| 0.85—0.90 | 0.8710      | 0.5001      | 0.4740 | 0.4203 | 0.5117 | 0.5562 | 1.1944   |
| 0.90—0.95 | 0.9247      | 0.5677      | 0.5196 | 0.4543 | 0.6147 | 0.6511 | 1.2658   |
| 0.95—1.00 | 0.9743      | 0.5875      | 0.5629 | 0.5032 | 0.6616 | 0.6949 | 1.3066   |
| 1.00—1.05 | 1.0263      | 0.6481      | 0.6304 | 0.5598 | 0.7029 | 0.7627 | 1.3652   |
| 1.05—1.10 | 1.0792      | 0.6982      | 0.6777 | 0.6249 | 0.7554 | 0.8310 | 1.4228   |
| 1.10—1.15 | 1.1168      | 0.7476      | 0.7191 | 0.6388 | 0.7901 | 0.8661 | 1.4580   |



The author (*op. cit.*) has derived values of equation (1) for the joints of the male cheliped. Values for the female joints have similarly been calculated and are as follows :—

$$\begin{aligned}\text{Is.} & \quad y = 1.3792x^{(0.40667+0.05239x)} e^{-0.10477x} \\ \text{Me.} & \quad y = 1.1438x^{(0.43296+0.05178x)} e^{-0.09785x} \\ \text{Ca.} & \quad y = 0.8213x^{(0.57277+0.02915x)} e^{-0.05685x} \\ \text{Pro.} & \quad y = 0.9502x^{(0.58641+0.04392x)} e^{-0.07943x} \\ \text{Dac.} & \quad y = 1.8849x^{(0.27958+0.08056x)} e^{-0.15102x} \\ \text{Cheliped} & \quad y = 6.0652x^{(0.42464+0.05121x)} e^{-0.09154x}\end{aligned}$$

Table II gives the values of  $\rho$  calculated from equation (2).

TABLE II

*The values of  $\rho$  for the second cheliped and its segments for the 13 values in the male and 12 values in the female of the group average carapace length (denoted as  $x$ )*

| $x$                 | $\rho$ |      |      |      |      |          |
|---------------------|--------|------|------|------|------|----------|
|                     | Is.    | Me.  | Ca.  | Pro. | Dac. | Cheliped |
| $\delta$ (I Phase)* |        |      |      |      |      |          |
| 4.9                 | 0.73   | 0.79 | 1.04 | 0.53 | 0.52 | 0.76     |
| 5.4                 | 0.75   | 0.83 | 1.01 | 0.64 | 0.62 | 0.80     |
| 6.0                 | 0.77   | 0.86 | 0.99 | 0.74 | 0.73 | 0.84     |
| 6.7                 | 0.80   | 0.91 | 0.95 | 0.90 | 0.89 | 0.91     |
| 7.5                 | 0.84   | 0.97 | 0.90 | 1.09 | 1.09 | 0.99     |
| 8.5                 | 0.89   | 1.06 | 0.84 | 1.36 | 1.37 | 1.11     |
| 9.4                 | 0.94   | 1.14 | 0.78 | 1.61 | 1.63 | 1.21     |
| 10.7                | 1.01   | 1.26 | 0.69 | 1.97 | 2.01 | 1.37     |
| $\delta$ (II Phase) |        |      |      |      |      |          |
| 11.9                | 0.89   | 0.64 | 0.92 | 1.62 | 1.50 | 1.86     |
| 13.2                | 1.01   | 1.06 | 1.24 | 1.92 | 1.75 | 1.84     |
| 15.1                | 1.18   | 1.66 | 1.70 | 2.35 | 2.11 | 1.82     |
| 16.6                | 1.34   | 2.21 | 2.11 | 2.74 | 2.43 | 1.80     |
| 18.0                | 1.48   | 2.73 | 2.50 | 3.11 | 2.73 | 1.78     |
| $\phi$              |        |      |      |      |      |          |
| 3.9                 | 0.48   | 0.53 | 0.62 | 0.68 | 0.43 | 0.54     |
| 4.3                 | 0.51   | 0.56 | 0.64 | 0.71 | 0.49 | 0.58     |
| 4.7                 | 0.54   | 0.60 | 0.66 | 0.74 | 0.54 | 0.61     |
| 5.5                 | 0.61   | 0.66 | 0.69 | 0.80 | 0.64 | 0.68     |
| 6.0                 | 0.65   | 0.71 | 0.72 | 0.84 | 0.72 | 0.73     |
| 6.9                 | 0.74   | 0.80 | 0.77 | 0.93 | 0.87 | 0.83     |
| 7.4                 | 0.80   | 0.86 | 0.80 | 0.98 | 0.96 | 0.89     |
| 8.4                 | 0.90   | 0.97 | 0.86 | 1.07 | 1.13 | 1.00     |
| 9.4                 | 1.02   | 1.09 | 0.93 | 1.18 | 1.32 | 1.13     |
| 10.6                | 1.16   | 1.24 | 1.01 | 1.31 | 1.55 | 1.28     |
| 12.0                | 1.34   | 1.42 | 1.11 | 1.47 | 1.84 | 1.47     |
| 13.1                | 1.48   | 1.57 | 1.19 | 1.60 | 2.07 | 1.62     |

\* It has been mentioned before by the author (*op. cit.*) that there is a change in phase in growth relationship in the male cheliped.

Values of the equilibrium constant  $\alpha$  of the simple allometry equation and the means of the values of  $\rho$ , as given in Table II, were calculated and the results are shown in Table III.

TABLE III

*The values of  $\alpha$  (columns 1) and mean  $\rho$  (columns 2) for the cheliped and its joints (male and female)*

|            | Is.  |      | Me.  |      | Ca.  |      | Pro. |      | Dac. |      | Cheliped |      |
|------------|------|------|------|------|------|------|------|------|------|------|----------|------|
|            | (1)  | (2)  | (1)  | (2)  | (1)  | (2)  | (1)  | (2)  | (1)  | (2)  | (1)      | (2)  |
| ♂ (I Ph.)  | 0.84 | 0.84 | 0.97 | 0.98 | 0.91 | 0.90 | 1.10 | 1.10 | 1.11 | 1.11 | 0.99     | 1.00 |
| ♀          | 0.83 | 0.85 | 0.89 | 0.92 | 0.81 | 0.83 | 1.00 | 1.03 | 1.01 | 1.04 | 0.92     | 0.95 |
| ♂ (II Ph.) | 1.16 | 1.18 | 1.59 | 1.66 | 1.64 | 1.69 | 2.29 | 2.35 | 2.06 | 2.10 | 1.82     | 1.82 |

It will be seen that, as anticipated by Huxley himself (*op. cit.*), the values of the equilibrium constant  $\alpha$  for the cheliped or for its segments are (within the limits of error due probably to the fluctuations of sampling and insufficient size of data) only the means of the values of corresponding  $\rho$  calculated for different ages, represented by  $x$ , of the prawns.

The values of  $\rho$  enable the existence of the growth-gradient to be seen very clearly. From the records of Table II lines have been drawn in Fig. 1(*a, b, c*) for the cheliped and its segments giving the values of  $\rho$  (along the  $y$ -axis) for different values of the carapace length  $x$  (along the  $x$ -axis) in males and females. (These may be compared with the dotted lines drawn in the same figure to show the distribution of growth potential by the method adopted by Huxley (*op. cit.*), Tazelaar (1930) and others, of taking the centres of homologous regions at equal distances along the  $x$ -axis and the corresponding values of  $\alpha$  along the  $y$ -axis).

It is seen that there is a progressive change in the successive values of the growth-coefficient. Thus, the first phase of growth in the male cheliped starts with a high point in Ca., which shifts towards the end of the phase to Me. In the female it shifts from Pro. to Me. The second phase of growth in the male cheliped starts with only one high point (at Pro.) and later another high point, though less steep than the first, develops (in Me.).

Also, the order of the values of  $\rho$  changes from (Dac., Pro., Is., Me. and Ca.) to (Ca., Is., Me., Pro. and Dac.) in the first phase of growth of the male cheliped, thus almost reversing itself. In the female the order changes from (Dac., Is., Me., Ca. and Pro.) to (Ca., Is., Me., Pro. and Dac.). Hence the final order of values of the growth-coefficients is identical in the two cases. This is interesting, for, as shown by the  $\alpha$  values in the above table and as pointed out by Huxley (*op. cit.*) and others, the growth pattern in the female cheliped is generally comparable with the one in male cheliped of most of the species of this genus in its first phase of growth. It will thus be seen that the growth-gradient, as exhibited by the  $\alpha$  values, does not indicate the final order of the values of the growth-coefficient for the segments of the cheliped. On the other hand, as the  $\alpha$  values are identical with the values of the mean  $\rho$ , it can be said to show a pattern obtained by pooling together of the patterns in the various stages of growth during a particular phase.

In the second phase of growth of the male cheliped the order of the values of  $\rho$  changes from (Me., Is., Ca., Dac. and Pro.) to (Is., Ca., Me., Dac. and Pro.).

Thus, it is seen that although the final order of values of the growth-coefficient may be the same in male (I Phase) and female chelipeds, the flow of the growth potential, before the final order is reached, has been different in the two cases. It may be noted here that Ca. decreases from a small positive allometric to a marked negative allometric joint in the first phase of growth in the male cheliped, while in the female it is just the opposite, i.e. it increases from marked negative allometry to a small positive one.

To study more clearly the difference in the growth behaviour of the male (I Phase) and the female chelipeds it will be interesting to calculate the values of  $\frac{dp}{dx}$  ( $= 2a+c+a \log_e x$ ). This has been done in Table IV.

TABLE IV

*The values of  $\frac{dp}{dx}$  for various values of the group average carapace length (x)*

| x                   | $\frac{dp}{dx}$ |       |        |       |       |          |
|---------------------|-----------------|-------|--------|-------|-------|----------|
|                     | Is.             | Mo.   | Ca.    | Pro.  | Dac.  | Cheliped |
| $\delta$ (I Phase)  |                 |       |        |       |       |          |
| 4.90                | 0.037           | 0.057 | -0.043 | 0.181 | 0.187 | 0.074    |
| 5.45                | 0.040           | 0.063 | -0.047 | 0.198 | 0.204 | 0.082    |
| 5.98                | 0.042           | 0.068 | -0.051 | 0.212 | 0.219 | 0.088    |
| 6.68                | 0.045           | 0.074 | -0.055 | 0.229 | 0.237 | 0.096    |
| 7.49                | 0.048           | 0.081 | -0.060 | 0.247 | 0.255 | 0.104    |
| 8.54                | 0.052           | 0.088 | -0.065 | 0.267 | 0.276 | 0.114    |
| 9.43                | 0.055           | 0.092 | -0.069 | 0.282 | 0.292 | 0.121    |
| 10.69               | 0.058           | 0.100 | -0.074 | 0.302 | 0.312 | 0.130    |
| $\delta$ (II Phase) |                 |       |        |       |       |          |
| 11.92               | 0.087           | 0.305 | 0.232  | 0.219 | 0.182 | -0.012   |
| 13.25               | 0.092           | 0.321 | 0.244  | 0.230 | 0.191 | -0.013   |
| 15.07               | 0.096           | 0.342 | 0.259  | 0.244 | 0.202 | -0.014   |
| 16.64               | 0.100           | 0.358 | 0.270  | 0.255 | 0.211 | -0.014   |
| 18.05               | 0.104           | 0.371 | 0.280  | 0.263 | 0.218 | -0.015   |
| $\eta$              |                 |       |        |       |       |          |
| 3.90                | 0.071           | 0.076 | 0.041  | 0.068 | 0.120 | 0.081    |
| 4.33                | 0.077           | 0.082 | 0.044  | 0.073 | 0.128 | 0.086    |
| 4.73                | 0.081           | 0.086 | 0.047  | 0.077 | 0.135 | 0.090    |
| 5.46                | 0.089           | 0.094 | 0.051  | 0.083 | 0.147 | 0.098    |
| 5.97                | 0.094           | 0.098 | 0.054  | 0.087 | 0.154 | 0.102    |
| 6.90                | 0.101           | 0.100 | 0.058  | 0.093 | 0.166 | 0.110    |
| 7.43                | 0.105           | 0.110 | 0.060  | 0.096 | 0.172 | 0.114    |
| 8.41                | 0.112           | 0.116 | 0.064  | 0.102 | 0.182 | 0.120    |
| 9.43                | 0.118           | 0.122 | 0.067  | 0.107 | 0.191 | 0.126    |
| 10.62               | 0.124           | 0.128 | 0.070  | 0.112 | 0.200 | 0.132    |
| 12.00               | 0.130           | 0.134 | 0.074  | 0.118 | 0.210 | 0.138    |
| 13.09               | 0.135           | 0.133 | 0.076  | 0.121 | 0.217 | 0.143    |

Fig. 2 (*a, b, c*) has been drawn to show the change in the value of  $\frac{d\rho}{dx}$  (taken along the *y*-axis) for the values of the carapace length *x* (taken along the *x*-axis).

Table V gives the mean values of  $\frac{d\rho}{dx}$  (as given in Table IV) for the male and the female segments.

TABLE V  
The mean values of  $\frac{d\rho}{dx}$  for the segments of the male and the female chelipeds

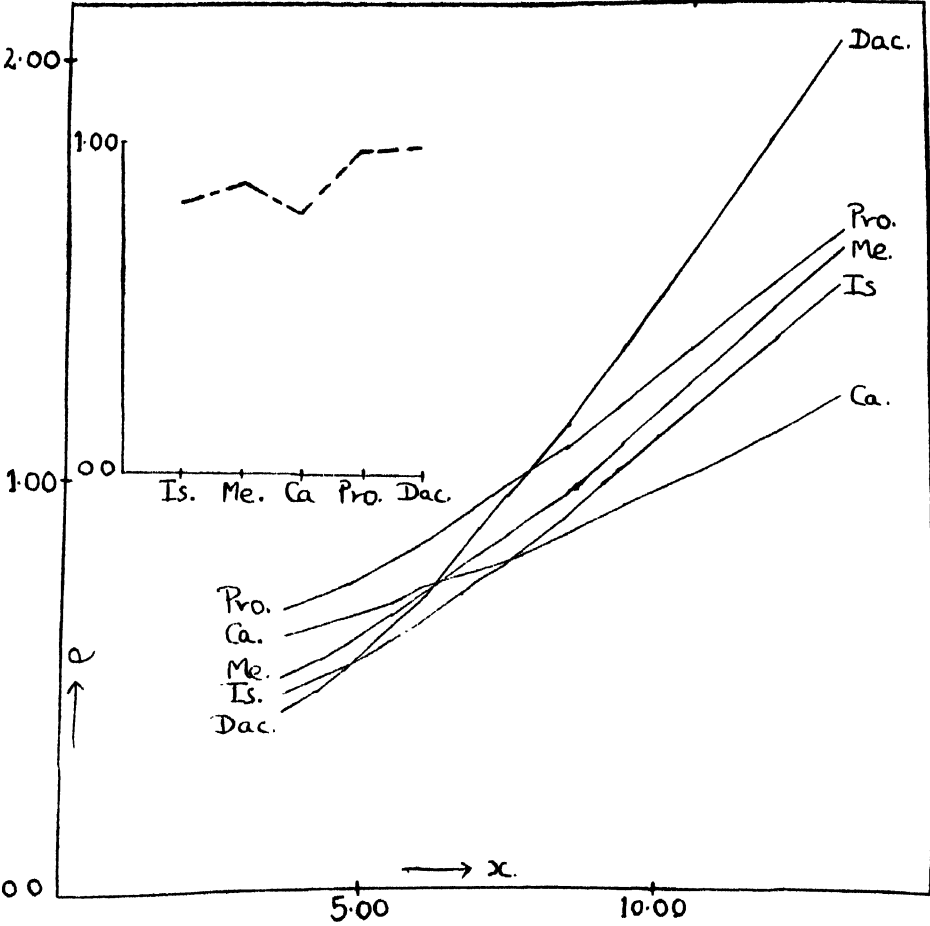
|              | Is.   | Mo.   | Ca.    | Pro.  | Dac.  | Cheliped |
|--------------|-------|-------|--------|-------|-------|----------|
| ♂ (I Phase)  | 0.047 | 0.078 | -0.058 | 0.240 | 0.248 | 0.101    |
| ♀            | 0.103 | 0.107 | 0.059  | 0.095 | 0.168 | 0.112    |
| ♂ (II Phase) | 0.096 | 0.339 | 0.257  | 0.242 | 0.201 | -0.014   |

These reflect clearly upon the significance of the shift of the growth-centre and also on the existence of it, as discussed earlier in this paper. From these it is seen that :

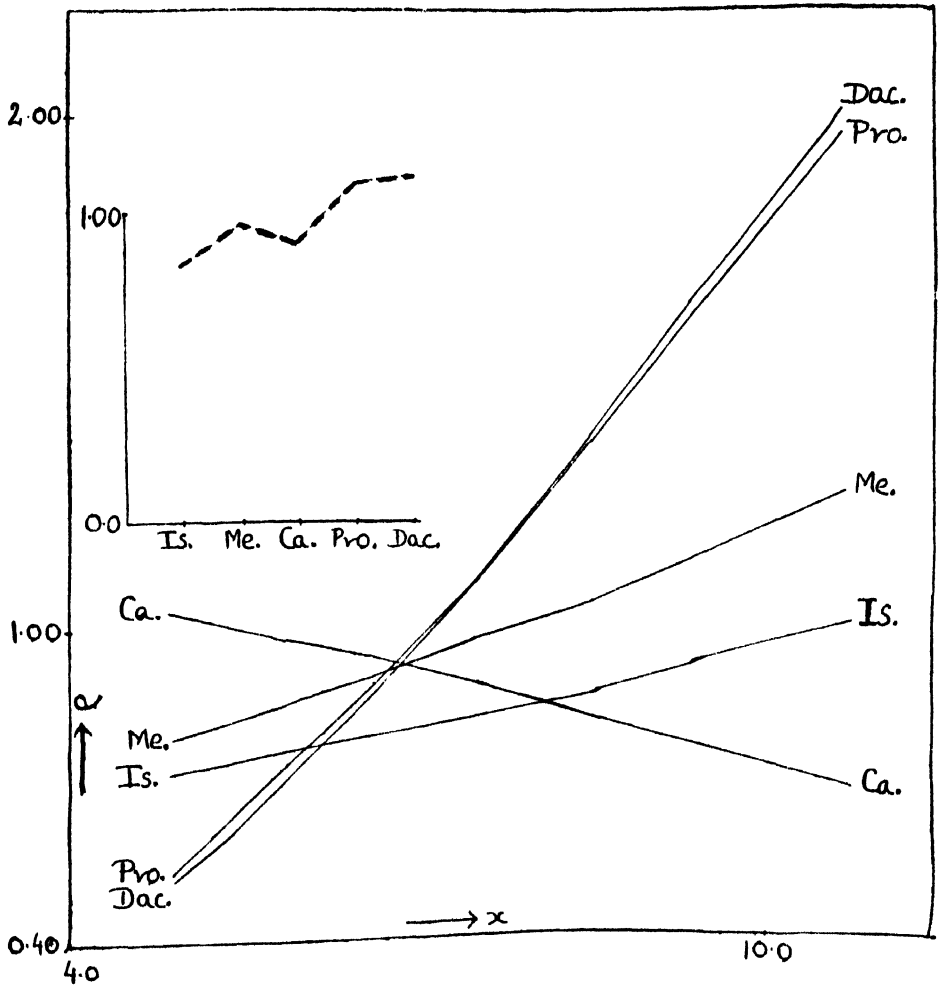
- (i) In the distal region of the cheliped consisting of the chela (Pro. and Dac.) the relative growth rate tends to increase faster in the male (I Phase) than in the female ; and it is just the opposite in the basal region (Is. and Me.) where the growth-coefficients for the male segments increase at lesser rates than what they do for the female ones.
- (ii) In general it is seen that the differences in the change of rates of growth between the male segments (I Phase) are much more marked than between the female segments. This reveals an important fact that the growth-gradient in the female cheliped is more stable than in the male cheliped.
- (iii) The rates at which the growth-coefficients increase in value follow different orders in male (I Phase) and female joints. Thus in the male it is (Ca., Is., Mo., Pro. and Dac.) and in the female it is (Ca., Pro., Is., Me. and Dac.) ; Ca. in the former case actually loses its rate of growth. In male (II Phase) the order is (Is., Dac., Pro., Ca. and Me.).
- (iv) Although the growth-centre is elsewhere, Dac. gains its final size at a faster rate than any other segment of the cheliped, both in the male (I Phase) and in the female. It thus forms the region of greatest activity in the cheliped. In the male (II Phase) it is Me. which gains its growth at the fastest rate of all the segments of the cheliped.

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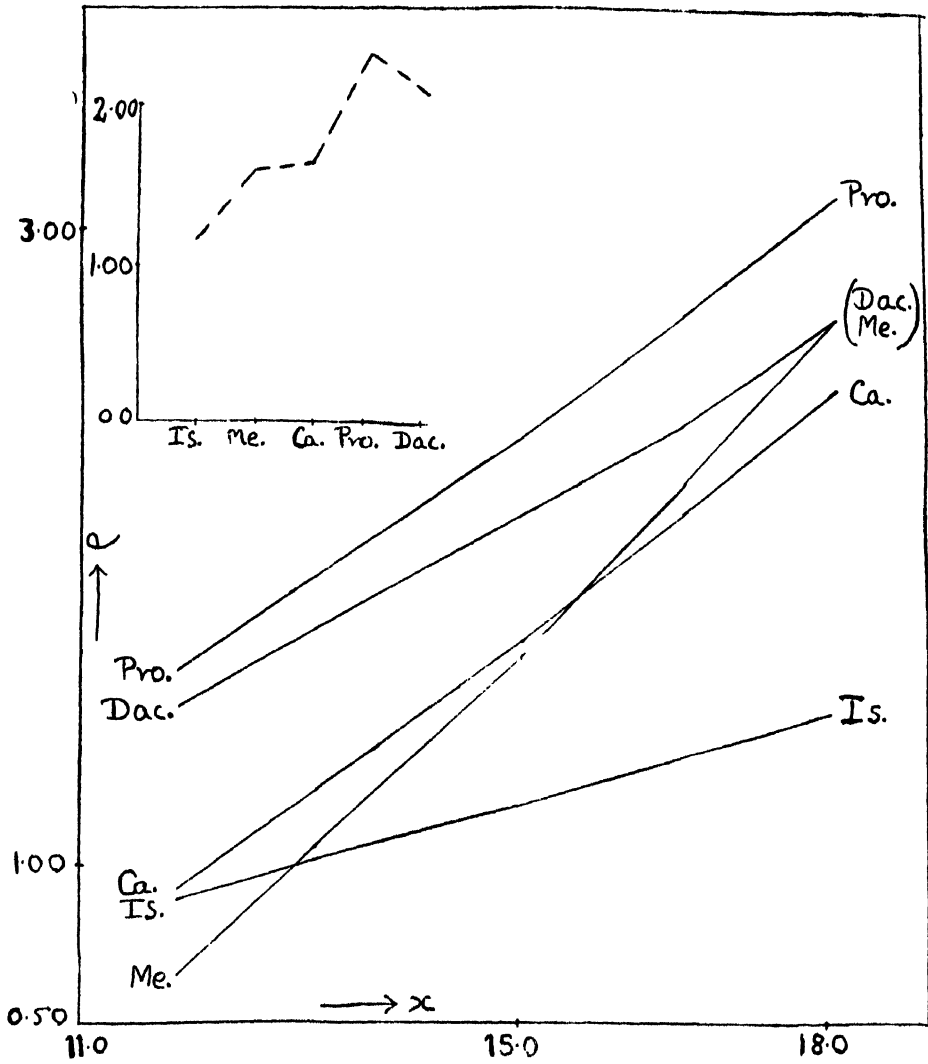
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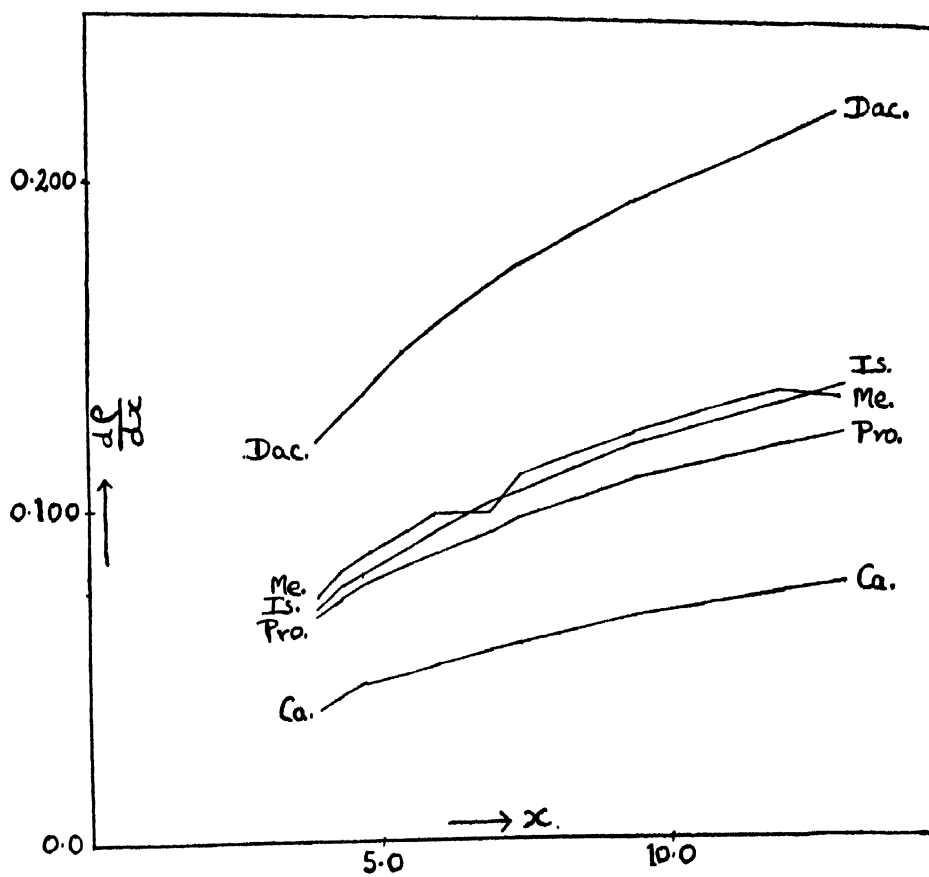
Text figure. 1(a). Showing growth-gradient within the cheliped (female).



Text-figuro. 1(b). Showing growth-gradient within the cheliped ( male, I Phase ).

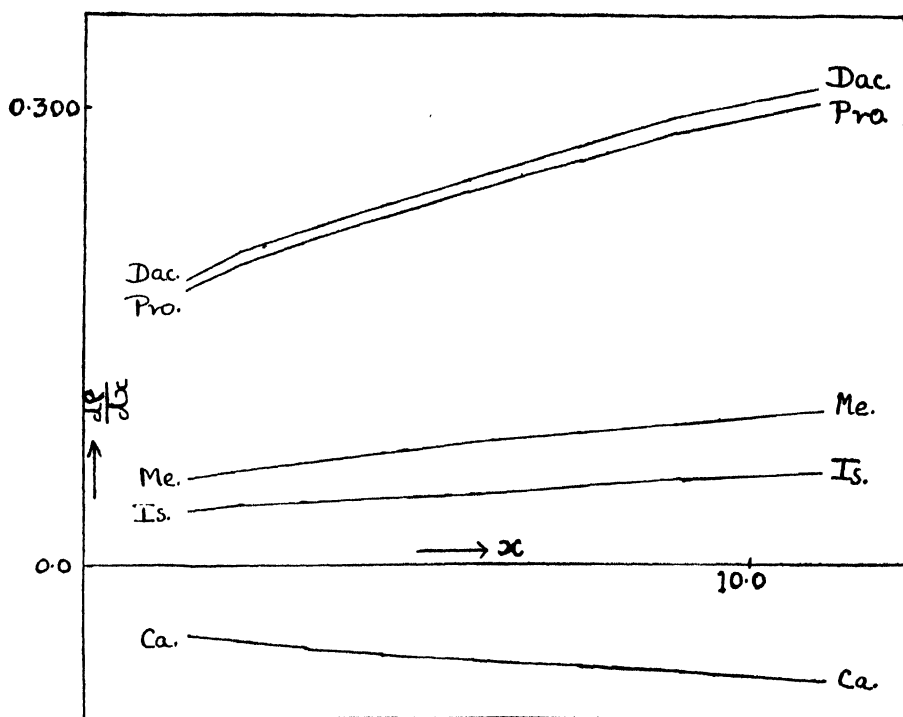


Text figure. 1(c). Showing growth-gradient within the cheliped (male, II Phase).

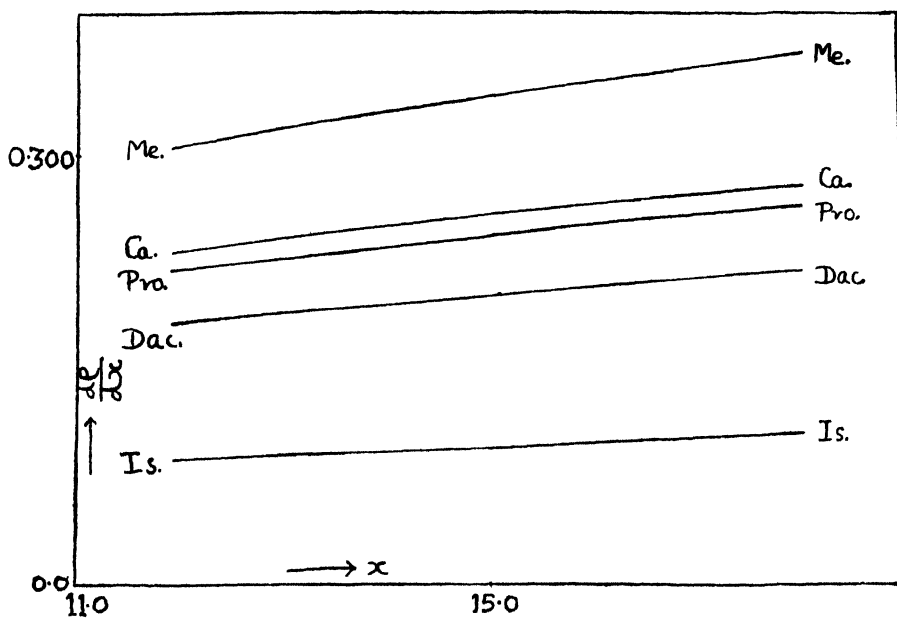


Text figure. 2(a). Showing the graph of  $\frac{dp}{dx}$  ( $y$ -axis) against the carapace length  $x$  ( $x$ -axis) ; female.





Text figure. 2(b). Showing the graph of  $\frac{d\rho}{dx}$  (y-axis) against the carapace length  $x$  (x-axis) ; male, I phase.



Text figure. 2(c). Showing the graph of  $\frac{d\rho}{dx}$  (y-axis) against the carapace length  $x$  (x-axis) ; male, II Phase,

GENUS *RICCIA* IN INDIA—II\*  
SPECIES OF *RICCIA* FROM SOUTH INDIA WITH DESCRIPTION OF  
A NEW SPECIES AND NOTES ON THE SYNONYMY OF SOME  
RECENTLY DESCRIBED ONES\*\*

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ABSTRACT

With a view to describe the liverwort flora of relatively little explored territories of India, the species of *Riccia* from South India have been worked out. In all eleven species have been recognized (Chopra, 1938 ; Udar 1957c ; Pandé and Udar, present communication) excluding *R. fluitans*-complex which will be treated separately (as it should now be understood to be from India) in view of the cytological and cultural work by Lorbeer (1934) and Muller (1940, 1941). A new species, *Riccia tuberculata* Pandé et Udar, has been discovered from South India. This species is characterised by distinctive tubercular thickenings on the cells of some of the assimilatory filaments, a peculiar feature known so far only in *R. bistrata* Evans but the pattern in the two species are different. Two of our little known species viz. *R. melanospora* Kash. and *R. plana* Taylor have been described in detail along with illustrations. A latin diagnosis of the former has also been included to complete its description. Two of the recently described species viz. *R. intermedia* Jones, from Tropical Africa and *R. bengalensis* Khan from East Pakistan have been shown to be synonyms of *R. discolor* L. et L. and *R. billardieri* Mont. et N. respectively. A key, for facilities of separation and identification of all the species now known from South India, has been given.

INTRODUCTION

With a view to work out the details of the Bryology of some of the relatively little explored Indian territories Pandé and Srivastava made extensive collections of hepatics and mosses during September–October, 1950, from several localities in South India. The hepatics in this collection are now being systematically worked out by the authors who hope to present a detailed account of the liverwort flora in due course based on the study of different genera represented in this collection. A part of the collection, containing specimens of *Riccia*, has been worked out and the study has revealed several interesting as well as new or little-known species from the country. Already *Riccia crozalsii* Lev. and *R. warnstorffii* Limpr. have been reported as new to Indian flora (Udar, 1957c) based on a critical examination of this collection. The other species include *R. melanospora* Kash., *R. discolor* L. et L., *R. billardieri* Mont. et N., *R. gangetica* Ahmad, *R. huebeneriana* Lindenb., *R. plana* Taylor and a species which differs markedly from all the known species of the genus and deserves a new specific rank.

Chopra (1938) has listed only three authentic species from South India viz. *R. cruciata* Kash., *R. frostii* Aust. and *R. fluitans* L. Of these, *R. fluitans* has been recently shown, through cytological and cultural investigations (Lorbeer, 1934 ; Müller, 1940, 1941), to be a complex of four species, the segregates being *R. fluitans* L. emend K. Müller, *R. canaliculata* Hoffm., *R. duplex* Lorb. and *R. rhenana* Lorb.

\* Part I of this series is due to appear in *J. Indian bot. Soc.*, **36**, No. 4, 1957.

\*\* Contribution from the Department of Botany, Lucknow University, India, New Series, No. 31.

In view of these observations *R. fluitans-complex* in India needs a careful reinvestigation and the authors hope to present a detailed discussion on this aspect soon in a separate communication.

Thus, eleven species of *Riccia* (excluding *R. fluitans-complex*) occur in South India (Chopra, 1938 ; Udar, 1957c ; Pandé and Udar, present communication). The key given below will facilitate their separation and identification. For the sake of convenience the division of the genus *Riccia* into subgenera *Euriccia* and *Ricciella* has been adopted.

1. Thallus with compact assimilatory zone and narrow air spaces  
Subgenus *Euriccia*.....3.
2. Thallus with loosely arranged assimilatory zone and wider air spaces. Subgenus *Ricciella*.....12.
3. (a) Plants monoecious.....4.
- (b) Plants dioecious.....10.
4. (a) Thalli ciliate.....5.
- (b) Thalli non-ciliate.....8.
5. (a) Spore black and perfectly opaque at maturity, 80–100 $\mu$  .....6.
- (b) Spore dark brown at maturity, 50–90 $\mu$  .....7.
6. Thallus bluish green, segments small, anteriorly sulcate, rest nearly flat, cilia small and broad, 100–150 $\mu$  long.....1. *R. melanospora*.
7. (a) Thallus yellow green, segments narrow, linear, apex rounded, deeply sulcate anteriorly and sulcus broader behind, cilia large, spore 50–80 $\mu$  .....2. *R. warnstorfi*.
- (b) Thallus bluish green, segments lanceolate, anteriorly deeply sulcate, rest very broadly channelled, spore 70–90 $\mu$  .....3. *R. crozalsii*.
8. (a) Spore winged.....9.
- (b) Spore unwinged.....11.
9. Thallus small, about 3 times broader than high, characteristic tubercular thickenings on the cells of the assimilatory filaments, spore irregularly reticulate or papillate, 80–110 $\mu$ , wing crenate.....4. *R. tuberculata*.
10. Male plants comparatively smaller than female, spore unwinged, reticulate, 80–100 $\mu$  .....5. *R. discolor*.
11. (a) Thallus large, 4–6 times broader than high, spore reddish brown, reticulate, 90–140 $\mu$ , angles of reticulations drawn out into prominent projections.....6. *R. billardieri*.
- (b) Thallus small, about 3 times broader than high, spore perfectly black and opaque at maturity, 8–16 small reticulations across the outer face, coarsely crenate in profile, 80–120 $\mu$  .....7. *R. gangetica*.
12. (a) Plants monoecious.....13.
- (b) Plants dioecious.....16.
13. (a) Thalli narrow repeatedly branched.....14.
- (b) Thalli very broad, cruciate or in rosettes.....15.
14. (a) Spore small, upto 60 $\mu$  .....8. *R. huebeneriana*.
- (b) Spore much larger, 80–110 $\mu$  .....9. *R. plana*.
15. Thallus as a rule cruciform, spore upto 70 $\mu$ , reticulations more or less complete.....10. *R. cruciata*.
16. Thalli forming well defined rosettes, female rosette normally larger than male, the latter usually pinkish, spore incompletely reticulate, upto 60 $\mu$  .....11. *R. frostii*.

#### 1. *Riccia melanospora* Kash.

*R. melanospora* is apparently one of our endemic species since it has not been reported so far from any other part of the world. The only published account

of this plant is its description by Kashyap (1929) which unfortunately is somewhat inadequate and lacks illustrations. Opportunity has, therefore, been taken to fill in this gap in our knowledge of this interesting plant and also to provide its latin diagnosis\*.

*R. melanospora* was instituted by Kashyap (1929) to include a specimen of *Riccia* from Hoshiarpur. Later it was also reported by him (Kashyap, 1932) from Lucknow, on the basis of the material sent to him by Pandé, and from Konsa Nag, 12,000 ft., Kashmir, on a collection sent to him by Dr. A. C. Joshi. In the present paper this species is being recorded for the first time from South India and possibility should not be excluded that the species may have a wider range of distribution and it may some day be discovered from some of the neighbouring countries.

Monoecious, bluish green; thalli small, simple or once furcate, in overlapping patches, occasionally forming distinct rosettes; ciliate, cilia 100–150 $\mu$  long, hyaline or pink, present both on margins and on surface; lobes oblong, upto 5 mm. long and 2 mm. broad; sulcate anteriorly and posteriorly broad, flat or broadly concave; air spaces narrow, assimilatory zone compact; *epidermal cells* rounded or oval, hyaline; ventral surface projecting prominently, hyaline in the middle but deep purple below the wings; *cross-section* semi-circular; about two times broader than high or as broad as high; *scales* large, hyaline or deep purple; sex organs in 1–4 rows, papillae very slightly projecting above; *sporophytes* bulge dorsally, occasionally developed near the margin of the thallus; *spore* dark and perfectly opaque at maturity, 80–100 $\mu$  in the maximum diameter, often much smaller, reticulate with 8–16 reticulations across the outer face, about 3.3 $\mu$  wide; winged, wings upto 6.6 $\mu$ , usually imperfectly developed, interrupted, quite often absent, tri-radiate mark conspicuous.

*Coll.* Pandé and Srivastava. *Loc.* Govt. Bot. Garden, Ootacamund. *Habitat* : Growing on red soil. *Date* : October 7, 1950. Pandé collection No. 4723, Lucknow University. (Text fig. 1).

*R. melanospora* is one of our xerophytic species and generally occurs on exposed places favouring particularly gravel foot paths. Under intense sun, when the atmosphere gets drier after monsoons, the wings are upturned exposing the deep purple scales.

The perfectly black and opaque colour of the spore masks the surface reticulations and also the wing but a treatment with dilute nitric acid for about half an hour brings out the details clearly. Prolonged treatment should, however, always be avoided.

In its dark opaque spores and larger number of reticulations *R. melanospora* approaches *R. gangetica* but the latter is non-ciliate, with thalli distinctly channelled and spore larger and unwinged.

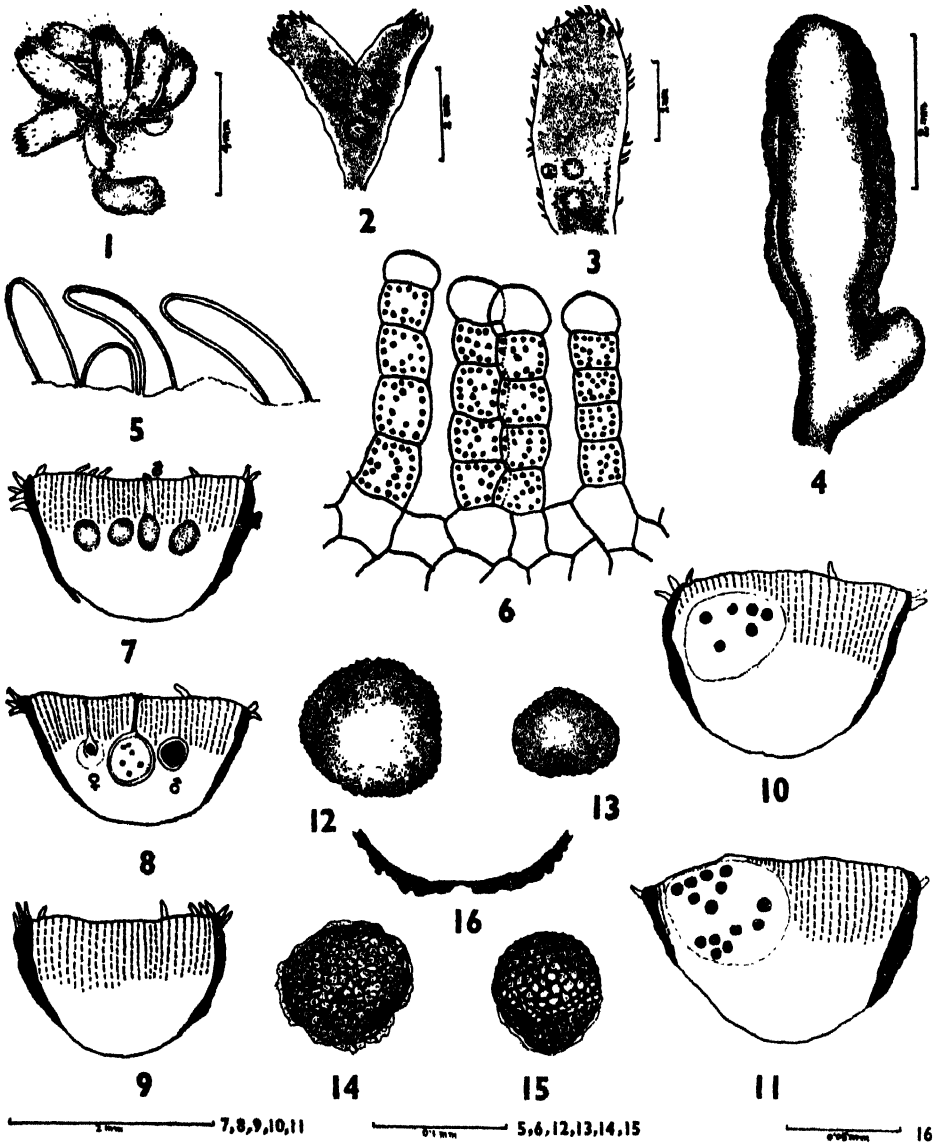
## 2. *Riccia warnstorffii* Limpr.

*R. warnstorffii* is certainly a luxuriantly represented species in South India although this is known so far only from this territory in India. An illustrated account of this has been already given by Udar (1957c) along with *R. crozalsii*. These two species are very recent additions to our hepatic flora.

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\* *Monoica*, parva, coeruleo-viridis, plus minusve glauca; *frons* ad 5 mm. longa, ad 2 mm. lata, simplex vel bifurcata; lobis linearibus, apice minute emarginato et truncato; fronte sulcata, caeteris partibus planis vel convexis, marginibus acutis, facie antica  $\pm$  dense spinosa, spinis hyalinis et purpureis, tenerrimis, 100–150 $\mu$  longis; thalli sectio crassa, duplo latior quam alta; *squamae* purpureae, imbricatae, margines haud superantes; *sporae* 80–100 $\mu$  fuscae, reticulatae, papillatae, reticulationibus 3.3 $\mu$  latis, angulis papillarum erectis; alata, ala regulari et brevi, 3.3–6.6 $\mu$ . lata,

*Coll.* Pandé and Srivastava. *Loc.*: On way to Coonoor. *Habitat*: Growing on red soil. *Date*: October 7, 1950. Pandé Collection No. 3802; *Loc.*: Govt. Botanical Garden Ootacamund. *Date*: October 9, 1950. Pandé collection No. 3121, 3123, 3815 3817, 5124, 5125.



TEXT-FIG. 1. *Riccia melanospora* Kash.

Fig. 1. Habit. 2. Branched thallus. 3. A thallus with a sporophyte at the margin. 4. Thallus, ventral. Note conspicuous scales. 5. Cilia. 6. Cross section of thallus showing assimilatory filaments and epidermis. 7-9. Cross section of a thallus at apex, in the middle and at the base respectively. 10-11. Cross sections showing superficial marginal sporophytes. 12-13. Dark and opaque spores. Note the disparity in size. 14. Spore, outer face. 15. Spore, inner faces. 16. Wing magnified.

3. *Riccia crozalsii* Lev.

*R. crozalsii* is a rather extremely rare species to come across and only one rosette is represented in the collection. In this country so far this species is known only from S. India and an illustrated account has already been given by Udar (1957c) from a part investigation of the South Indian hepatics in Pandé Collection.

*Coll.* Pandé and Srivastava. *Loc.*: Govt. Botanical Garden, Ootacamund, *Habitat*: Growing on red soil. *Date*: October 9, 1950. Pandé Collection No. 3814, Lucknow University.

4. *Riccia tuberculata* Pandé et Udar, *sp. nov.*

Monoica, glauco-virens; *frons* ad 5 mm. longa, ad 2 mm. lata, simplex vel bifurcata, lateribus ascendentibus, marginibus acutis, sulcus ad apicem profundus et acutus; *squamae* magnae, integrae, imbricatae, atro-purpureae, marginem superantes; *sporae* 80–110 $\mu$ , brunneae, conferte papillatae et irregulariter lamellatae, ad angulos papillatae, facies internae plus minusve regulariter lamellatae; anguste alatae, margo alae papillatae, 6.6 $\mu$ .

Monoecious, bluish green; *thallus* upto 5 mm. long, 2 mm broad, simple or once furcate, overlapping or isolated, often forming rosettes; *segments* ovate-linear, prominently convex ventrally, apex rounded or acute, dorsally sulcate, sulcus prominent anteriorly; wings more or less convex, margin acute and ascending; *scales* large, overlapping, dark purple, *conspicuously extending beyond the margins*; *epidermal cells* oval-papilliform, hyaline; air-spaces narrow; *assimilatory filaments* compact with characteristic tuberculate thickenings noticeable on the walls of the cells of some of these, thickenings extending upto the epidermal cells from the base of the assimilatory filaments and occasionally also present on some cells of the lower compact zone; antheridia and archegonia in the mid-dorsal line in a row, papillae projecting above the surface; *sporophytes* uniseriate, bulging prominently on the dorsal surface; *spore* dark brown 80–110 $\mu$  along the maximum diameter, *densely papillate to irregularly reticulate on the outer face*, more or less regularly reticulate on the inner faces, tri-radiate mark conspicuous; winged, wings upto 6.6 $\mu$  wide having a warty surface and crenulate margin.

*Coll.* Pandé and Srivastava. *Loc.*: Runnymede, Madras State, South India. *Habitat*: Growing on red soil. *Date*: October 6, 1950. Pandé Collection No. 3803, Lucknow University. (Text fig. 2).

The characteristic band of thickening found on the walls of the green cells represents a feature noted earlier only in the case of *R. bistrata* (Evans, 1919). The pattern of thickenings of *R. tuberculata* Pandé et Udar is, however, very different from *R. bistrata*. In the latter the band of thickening is uniform while in *R. tuberculata* it is distinctly tuberculate. Besides, the two species differ in sexuality, vegetative features and characters of spore as well.

The specimen of *Riccia* from South India has, therefore, been referred to a new species, *Riccia tuberculata* Pandé et Udar, *sp. nov.*

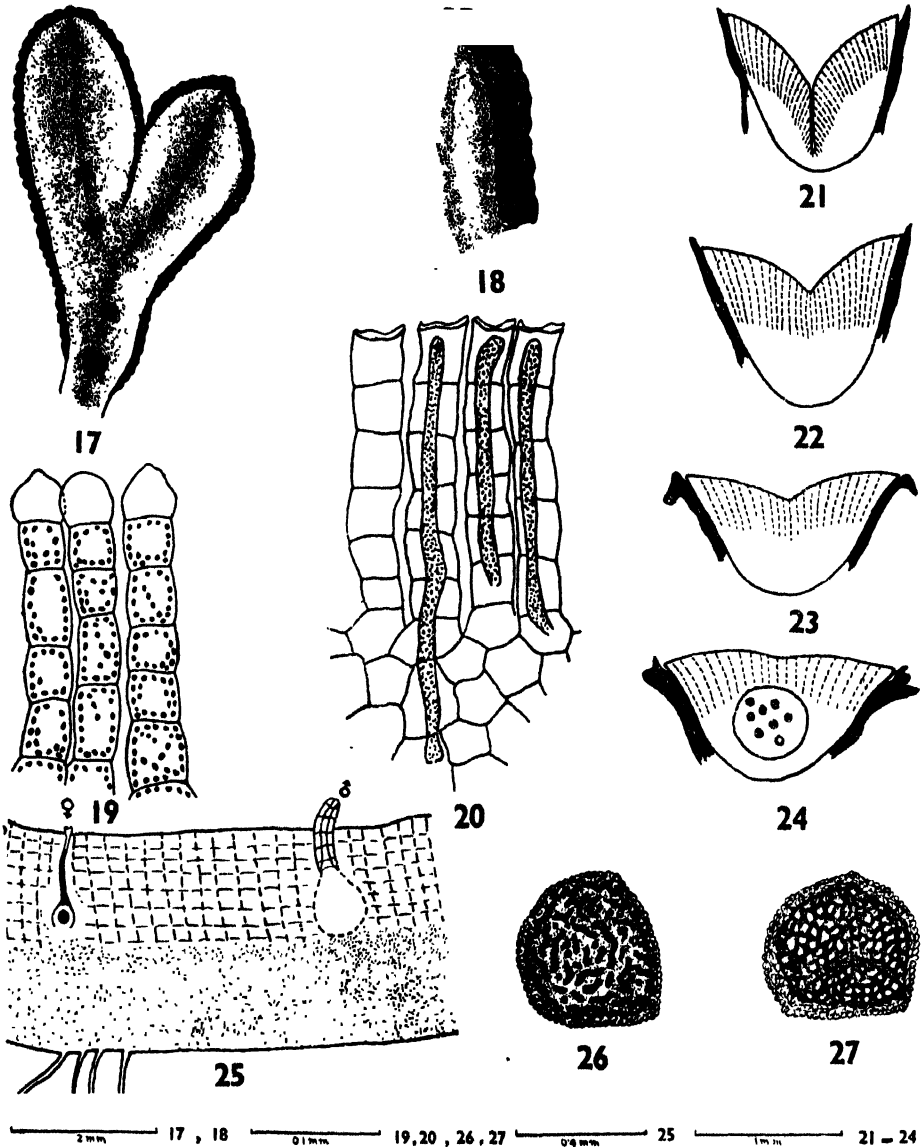
5. *Riccia discolor* L. et L.

A detailed description and a critical discussion of *R. discolor* has been presented in two earlier communications (Udar, 1957a; Pandé and Udar, 1957).

Recently Jones (1957) described a new *dioecious* species from Tropical Africa, *R. intermedia* Jones, which is apparently identical with *R. discolor*.

Through the courtesy of Dr. E. W. Jones beautiful type specimens of *R. intermedia* were obtained and on examination the authors have failed to notice any significant feature which would separate it from *R. discolor*. Dr. Jones had the

obvious difficulty as he could not secure authentic published accounts of *R. discolor* and, in his excellent paper, he makes a reference to the difficulty he experienced in not being able to compare his Tropical African Riccias with Indian species. It has since been possible for the authors to present a detailed account of this species



TEXT-FIG. 2. *Riccia tuberculata* Pandé et Udar.

Fig. 17. Thalls, dorsal. 18, Same, ventral. Note large prominent scales which almost coalesce. 19. Thallus in cross-section showing assimilatory filaments with epidermal cells. 20. Same showing characteristic tubercular thickenings. 21-24. Cross sections of thallus at the apex, behind the apex, in the middle and at the base, respectively. 25. V.L.S. of thallus showing its monoecious character. ♂, antheridium; ♀, archegonium. 26. Spore, outer face. 27. Spore, inner faces.

(Pandé and Udar, 1957). Since India and Africa are tropical countries and geographically largely identical, the possibility of finding common species in these two countries becomes all the more probable.

In a recent paper on the Ricciaceae of East Pakistan, Khan (1957) has described *R. discolor* as monoecious. This species is *strictly dioecious* (see Udar, 1957a; Pandé and Udar, 1957) and always distinct male and female thalli have been found in the Pandé Collection from practically all parts of India including the neighbouring territory of West Bengal. The plants usually grow intimately mixed with a monoecious species, *R. billardieri*, and a good deal of confusion becomes evidently possible if the plants are not carefully isolated for study. A reinvestigation of the specimens of *Riccia* described by Khan (1957) from East Pakistan, thus, becomes necessary in the light of the above observations.

*Coll.*: Pandé and Srivastava. *Loc.*: Mangalore. *Habitat*: Growing on red soil. *Date*: October 13, 1950. Pandé Collection No. 5495a, Lucknow University.

#### 6. *Riccia billardieri* Mont. et N.

*R. billardieri* is one of the most widely distributed species in South India. Recently it has been worked out with respect to its synonymy and taxonomic details by Udar (1957a) and Pandé and Udar (1957), cytology by Udar and Chopra (1957) and sporeling germination by Udar (1957b).

In a recent paper Khan (1957) has described *R. bengalensis* Khan from Dacca, East Pakistan. The vegetative features of this species as well as the description of the spore strongly answers to *R. billardieri*, a very common species known from the adjoining territory of West Bengal. The species according to Khan (1957) is characterised by the presence of prominently projecting sex papillae which occur in two or three rows. These papillae are exactly alike in *R. billardieri* (see Udar, 1957a, Fig. 6) as well and the authors feel certain that both these specimens are identical.

*Coll.*: Pandé and Srivastava. *Loc.*: Mahe. *Habitat*: Growing on red soil. *Date*: October 11, 1950. Pandé Collection No. 4753, Lucknow University; *Loc.*: Mangalore. Growing mixed with *Cyathodium* sp. *Date*: October 13, 1950. Pandé Collection No. 4595, 4597, Lucknow University.

#### 7. *Riccia gangetica* Ahmad.

In South India *R. gangetica* is apparently not so common as *R. discolor* and *R. billardieri*. A detailed account of this species and a discussion about its specific validity has been given by Udar (1957a) and Pandé and Udar (1957). The chromosome number and the origin of this species has been discussed by Udar and Chopra (1957) and Chopra and Udar (1957).

The specimens from South India are fertile but are extremely small in size being only 2 mm. long and about 0.5 mm. broad.

*Coll.*: Pandé and Srivastava. *Loc.*: Addenley Station. *Habitat*: Growing on red soil. *Date*: October 6, 1950. Pandé Collection No. 5107, Lucknow University.

#### 8. *Riccia huebeneriana* Lindenb.

*R. huebeneriana* was first reported from India by Udar (1956) on the basis of a collection made by Pandé (from Darjeeling on way to Badampton) and another by Mr. Singhal (from Kisli forest, Madhya Pradesh). This is the first record of this species from South India where it is apparently rare.



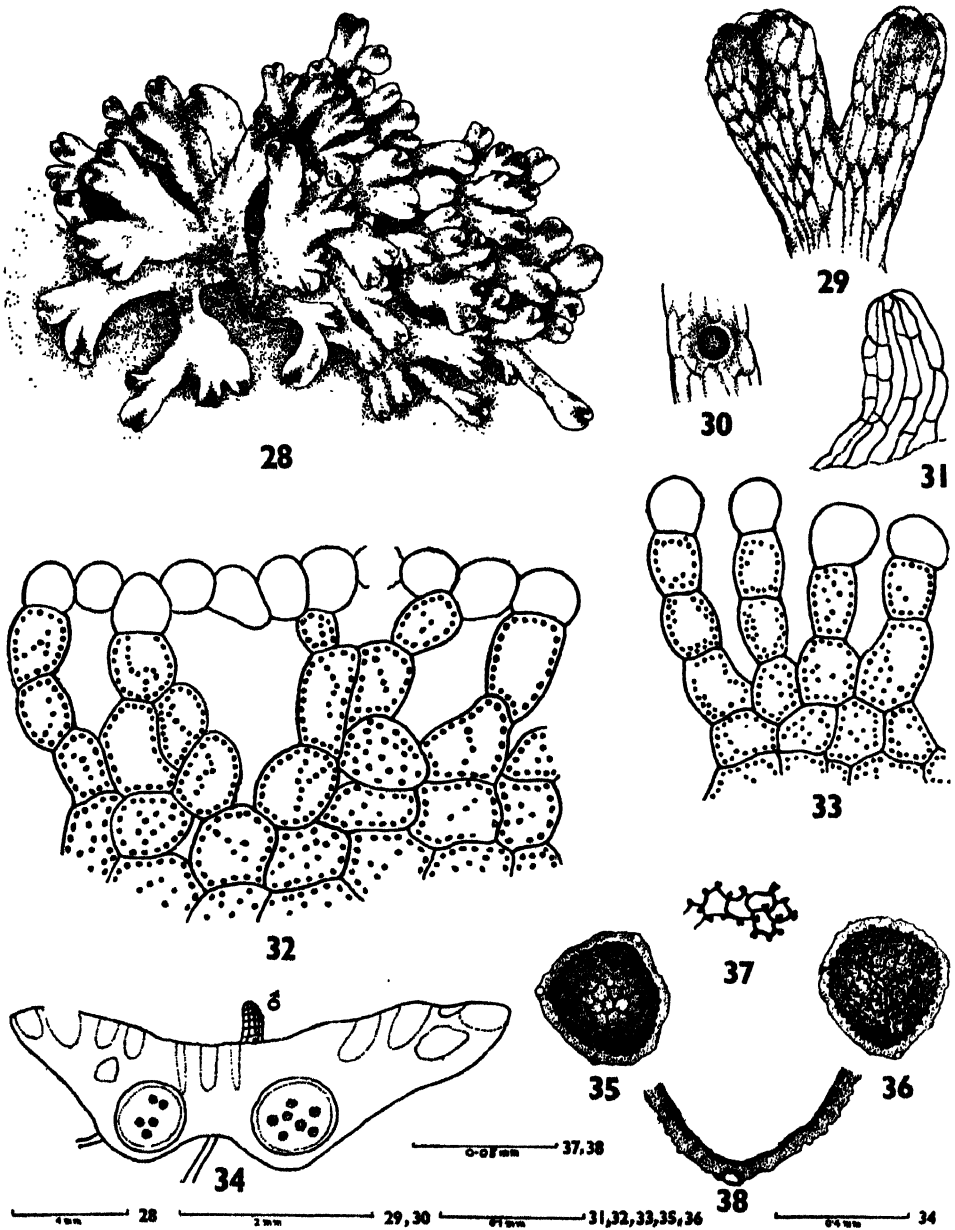
TEXT-FIG. 3 *Riccia plana* Taylor.

Fig. 28. Habit. 29. Areolate appearance of thallus. 30. A part of thallus showing ventrally projecting sporophyte, ventral view. 31. Antheridial papilla. 32, 33. Cross-section of thallus (enlarged) at the margin and in the middle respectively to show assimilatory zone and air spaces. 34. Cross-section of thallus at the point of dichotomy showing two ventrally projecting sporophytes and antheridial papilla projecting dorsally. 35. Spore, outer face. 36. Spore, inner faces. 37. Spore reticulations magnified. 38. Wing of the spore magnified.

*Coll.*: Pandé and Srivastava. *Loc.*: Compound of Mangalore School. *Habitat*: Growing on red soil. *Date*: October 6, 1950. Pandé Collection No. 5879, Lucknow University.

This species is likely to be confused with *R. plana* Taylor. The spores in the latter are, however, much larger.

#### 9. *Riccia plana* Taylor.

The Indian specimen of *R. plana* was originally referred to a new species of *Riccia*, *R. mangalorica* by Ahmad (Ahmad, 1942), who remarked that it may only be a variety of *R. plana*. Careful examination of the specimen of this plant has shown that it need not be segregated from *R. plana* (Udar, 1957a). As the only description of this liverwort, in literature on Indian Bryology, is based on a note by Ahmad (1942), a detailed account is presented below.

Monoeocious, grey-green; *thalli* several times dichotomously branched, isolated or densely crowded and overlapping, occasionally forming rosettes; loosely arranged assimilatory zone with wide air-spaces; ventral surface concolorous, rhizoids simple and tuberculate, tubercles not prominent; *scales* hyaline and difficult to isolate; *thallus* in *cross section* usually 4–5 times broader than high, extremely variable depending on the size of *thalli*; antheridial ostioles conical, hyaline and transparent, about  $250\mu$  above *thallus* surface; *sporophytes* projecting prominently on ventral surface; *spore* light brown to dark brown, tetrahedral,  $80\text{--}110\mu$  along the maximum diameter, reticulate with 6–10 reticulations across the outer face, reticulations  $7\text{--}10\mu$  wide, inner faces more or less incompletely reticulate, angles of reticulations project in the form of spines which may be truncate, entire or bifid, papillate in profile; winged, wing  $3.3$  to  $6.6\mu$  wide, broader at angles where occasionally an irregular pit or perforation occurs, wing margin finely erose.

*Coll.*: Pandé and Srivastava. *Loc.*: Compound of Mangalore Girls' School. *Habitat*: Growing on red soil. *Date*: October 6, 1950. Pandé Collection No. 5876 (fixed specimens), Lucknow University. (Text fig. 3).

This is one of the common species in South India.

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\* Not seen in original.

*Issued April 26, 1958.*

## EFFECTS OF GIBBERELLIC ACID ON THE FLOWERING OF *SESAMUM INDICUM* L.

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(Communicated by G. P. Majumdar, F.N.I.)

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### ABSTRACT

Gibberellic acid (GA) in the concentrations of 1, 10 and 100 ppm. of aqueous solution was applied as pre-sowing soaking treatment of seeds and also as foliar sprays and drops of different durations at three stages in the life-cycle of *Sesamum*, an annual plant with a single phase of growth. In none of the treatments, however, there was any early induction of flowering. On the contrary, a significant delay in the process was recorded when plants both 10 and 24 days old received a repeated treatment with 100 ppm. of GA.

It appears that the chemical has no florigenic property and its effect in bringing about an early termination of the vegetative cycle in certain annual and biennial plants with two distinct phases of growth,—one of rosette formation and the other of stem elongation and flowering, is secondary.

### INTRODUCTION

In recent years a number of papers has appeared dealing with growth and flowering responses of plants subjected to the treatments of gibberellins and gibberellic acid (Brian and Grove, 1957 ; Bukovac and Wittwer, 1957 ; Bünsow and Harder, 1956*a, b*, 1957 ; Gray, 1957 ; Lang, 1956, 1957 ; Marth *et al.*, 1956, Wittwer and Bukovac, 1957*a, b* and Wittwer *et al.*, 1957). In addition to the general effect of bringing about an extension growth, these chemicals also bring about an earliness in flowering in a number of plants like carrot, lettuce, endive, mustard, cabbage, henbane etc. In almost all the plants referred above, there are two distinct phases of growth, one leading to the formation of a rosette of leaves and the other to that of stem elongation and flowering. As in these plants onset of the reproductive phase and stem elongation go hand in hand, the effect of gibberellic acid in bringing about an earliness in flowering might be an indirect one i.e., by the way of causing an extension growth of the stem. In order to subject the above suggestion to experimental test, a detailed investigation has been undertaken with *Sesamum indicum* L. which is an annual plant with a single phase of growth.

### EXPERIMENTAL PROCEDURE

Two sets of experiments were carried out. In the first, 1, 10 and 100 parts per million of aqueous solution of gibberellic acid\* was applied as 12 hours' pre-sowing soaking treatment of the seeds. In the second, these solutions were applied as foliar sprays to runoff and as droppings on the leaf whorl. Spraying treatments were commenced when the plants were 10 and 24 days old, while that

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\* Sample of gibberellic acid used in this experiment was obtained through the kind courtesy of Eli Lilly and Company, Indianapolis, Ind., USA.

of dropping at an age of 30 days. In the case of 10 day old plants, the chemical was applied once in the life-cycle and also five times at both daily and weekly intervals. In plants 24 day old, five sprayings were done at the interval of three days and the rest two treatments were the same as in the previous case. Dropping on the leaf whorl was done by delivering approximately 0.05 c.c. of the solution with a micropipette.

Seeds of the variety T. 10 were sown in 9" pots containing well manured garden soil. Four pots were allotted to each of the variables with a final stand of three to four plants per pot. Dates of opening of the first flower from the first-formed bud were recorded for individual plants and the average time taken for anthesis by the plants of each treatment calculated. Number of leaves developed on these plants below the node of floral initiation including the cotyledonary ones has also been recorded.

The method of analysis of variance has been used to determine the statistical validity of the experiments.

### RESULTS AND DISCUSSIONS

Data collected have been presented in Tables I and II.

TABLE I

*Effect of 12 hours' pre-sowing soaking treatments of seeds of Sesamum indicum L. with different concentrations of gibberellic acid (GA) on the time taken for anthesis and the number of leaves at floral initiation. Number of plants are shown within brackets.*

Date of sowing : 11-7-57.

Error mean sq ; Anthesis : 0.32  
Leaf number : 0.97

| Concentration of GA | Time taken for anthesis | Earliness over control | Leaf No. | Decrease over control | Remarks                                  |
|---------------------|-------------------------|------------------------|----------|-----------------------|--|
| Control             | 33.66 days (15)         | —                      | 11.46    | —                     | None of the differences are significant. |
| 1 ppm.              | 33.53 " (15)            | 0.13                   | 10.66    | 0.80                  |  |
| 10 "                | 33.66 " (15)            | 0.06                   | 10.93    | 0.53                  |  |
| 100 "               | 33.66 " (15)            | 0.00                   | 11.20    | 0.26                  |  |

It would be seen that in none of the treatments there has been an early induction of flowering. On the contrary, when gibberellic acid in the concentration of 100 ppm. was sprayed once a week for five times commencing at an age of ten days and also daily for five consecutive days and five times once every third day commencing at an age of twenty four days, there has been a significant delay in the time taken for anthesis and also a significant increase in the number of leaves developed prior to floral initiation. Treatments given late in the life-cycle is more effective in bringing about this delay in flowering than the ones given early. In almost all the treated plants there was an extension growth due to elongation of the internodes.

Marth *et al.* (1956), while working with a number of plants failed to record any evidence that gibberellic acid could induce them to initiate flower primordia. On the other hand, both these authors and Gray (1957) found an inhibition of flower bud development in *Capsicum* for about 3 to 4 weeks after treatment with this chemical. Thimann (personal communication) could record no earliness in flowering in treated *Chenopodium* plants. Absence of response has also been noticed in onion by Rappaport (1956). Wittwer *et al.* (1957), while working with beans and tomatoes observed an early flowering in the early or strongly determinate

varieties without any reduction in the node number preceding the first flower and came to the conclusion that gibberellin did not specifically influence the flowering process.

Lindstrom *et al.* (1957) have reported hastening in flowering varying from 10 days to 4 weeks in annual plants like stocks, petunia, larkspur, English daisy, China aster and gerbera when grown during the fall and winter in the green house. However, as no record of the amount of vegetative growth expressed as leaf numbers preceding the first flowers has been taken, it is not possible to decide whether gibberellin did actually influence the process of flowering in these plants.

TABLE II

*Effect of different modes of application of gibberellic acid (GA) on the time taken for anthesis and the number of leaves at floral initiation of Sesamum indicum L. Number of plants are given within brackets.*

Date of sowing : 1-7-57.

Error mean Sq.; Anthesis : 13.1  
Leaf number : 4.8

| Age of commencement of treatment | Mode of application      | Conc. of GA | Time taken for anthesis in days | Earliness over control | Leaf No. | Decrease over control |
|----------------------------------|--------------------------|-------------|---------------------------------|------------------------|----------|-----------------------|
| Control                          |                          |             | 42.13 (15)                      | —                      | 12.66    | —                     |
| 10 days                          | Single spray             | 1 ppm.      | 41.72 (11)                      | 0.41                   | 12.54    | 0.12                  |
| " "                              | " "                      | 10 "        | 42.63 (11)                      | -0.50                  | 12.72    | -0.06                 |
| " "                              | " "                      | 100 "       | 42.72 (11)                      | -0.59                  | 12.72    | -0.06                 |
| " "                              | 5 sprays, once a day     | 1 "         | 24.46 (15)                      | -0.33                  | 12.40    | 0.26                  |
| " "                              | " " " "                  | 10 "        | 43.90 (10)                      | -1.77                  | 13.00    | -0.34                 |
| " "                              | " " " "                  | 100 "       | 43.25 (8)                       | -1.12                  | 12.75    | -0.09                 |
| 10 days                          | 5 sprays, once a week    | 1 ppm.      | 42.55 (9)                       | -0.42                  | 12.66    | 0.00                  |
| " "                              | " " " "                  | 10 "        | 44.25 (12)                      | -2.12                  | 12.66    | 0.00                  |
| " "                              | " " " "                  | 100 "       | †57.25 (8)                      | -15.12**               | 18.00    | -5.34**               |
| 24 days                          | Single spray             | 1 "         | 41.38 (13)                      | 0.75                   | 12.15    | 0.51                  |
| " "                              | " "                      | 10 "        | 41.23 (13)                      | 0.90                   | 12.00    | 0.66                  |
| " "                              | " "                      | 100 "       | 43.91 (12)                      | -1.78                  | 15.00    | -2.34                 |
| " "                              | 5 sprays, once a day     | 1 "         | 42.07 (14)                      | 0.06                   | 12.85    | -0.19                 |
| " "                              | " " " "                  | 10 "        | 43.00 (12)                      | -0.87                  | 13.66    | -1.00                 |
| " "                              | " " " "                  | 100 "       | 53.08 (12)                      | -10.95**               | 19.50    | -6.84**               |
| " "                              | 5 sprays, once in 3 days | 1 "         | 42.30 (13)                      | -0.17                  | 12.61    | 0.05                  |
| " "                              | " " " "                  | 10 "        | 42.63 (11)                      | -0.50                  | 12.18    | 0.48                  |
| " "                              | " " " "                  | 100 "       | 47.33 (9)                       | -5.20*                 | 18.44    | -5.78**               |
| 30 days                          | 5 drops, one per day     | 1 "         | 41.80 (10)                      | 0.33                   | 12.30    | 0.36                  |
| " "                              | " " " "                  | 10 "        | 42.30 (10)                      | -0.17                  | 12.40    | 0.26                  |
| " "                              | " " " "                  | 100 "       | 42.60 (10)                      | -0.47                  | 12.70    | -0.04                 |

† Several plants failed to flower.

\* Significant at 5 per cent level.

\*\* Significant at 1 per cent level.

There exists a fundamental difference in the growth characteristics of plants like *Sesamum* on the one hand and on the other the biennial and annual forms of plants like carrot, lettuce, endive, mustard, cabbage, henbane etc., which have in almost all the cases given a positive flowering response to gibberellic acid and gibberellin treatments. In the biennial and annual plants referred above, the process of flowering follows a rapid stem elongation after the formation of a rosette during the vegetative condition, while in *Sesamum* it is not so. Failure of gibberellic

acid to bring about an earliness in flowering in *Sesamum indicum* and a number of other plants suggests that this chemical does not have any florigenic property. It seems quite probable that induction of early flowering in these annual and biennial plants is brought about in an indirect way i.e., by causing the stems to elongate, a phenomenon which normally accompanies their flowering. Lang (1957) while working with several biennials, winter annuals, long-day and short-day plants, came to the conclusion that the effect of gibberellin on flower-formation might be a secondary one and the present findings support the view.

In several biennial plants gibberellic acid and gibberellins have been found to replace vernalization treatment of seeds (Blaney, 1957; Bukovac and Wittwer, 1957; Lang, 1957 and Wittwer and Bukovac, 1957c). However, this question does not arise in *Sesamum* as prechilling of seeds has no effect on its flowering (Chakravarti, 1956). It would be of great interest to determine the effect of gibberellic acid and gibberellins on the flowering of plants with a single phase of growth and giving a positive response to the vernalization treatment.

### SUMMARY AND CONCLUSIONS

In the present investigation effect of 1, 10, and 100 ppm. of aqueous solution of gibberellic acid (GA), when applied as pre-sowing soaking treatment of seeds and also as foliar sprays and drops of different durations at three stages in the life cycle of *Sesamum indicum* on its flowering behaviour has been studied. Data of the time taken for anthesis from the date of sowing and also of the amount of vegetative growth expressed as leaf numbers preceding the first flower have been collected.

In none of the cases, however, there has been, an early induction of flowering. On the contrary, in spraying treatments of 10 day old plants with 100 ppm. of GA once a week for five times and of 24 day old plants with the same concentration daily for five consecutive days and five times once every third day, there has been a significant delay in flowering as determined by both the criteria referred above.

It is concluded that GA does not have any florigenic property and it brings about an earliness in flowering in biennial and annual plants with two distinct phases of growth, one of rosette formation and the other of stem elongation and flowering, in an indirect way.

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CYTOLOGICAL STUDIES IN INDIAN MOSSES\*. III—*FUNARIA*  
*CALVESCENS* SCHW., *BRYUM CELLULARE* HOOK., *B.*  
*RAMOSUM* (HOOK.) MITT., *B. PSEUDO-*  
*PACHYTHECA* C. MÜLL., *POHLIA*  
*ELONGATA* HEDW. AND  
*P. FLEXUOSA* HOOK.

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ABSTRACT

The haploid number of chromosomes in *Funaria calvescens* Schw., *Bryum cellulare* Hook., *B. ramosum* (Hook.) Mitt., *B. pseudo-pachytheca* C. Müll., *Pohlia elongata* Hedw. and *P. flexuosa* Hook, is 14, 10, 10, 20, 11, 11, respectively.

INTRODUCTION

In the first paper of this series Pandé and Chopra (1957a) recorded their observations on the chromosome number in four Indian mosses, reporting the sex chromosomes in two of them viz. *Pogonatum stevensii* Ren. & Card. and *Bryum nitens* Hook. and subsequently (Pandé and Chopra, 1957b) the cytology of three more mosses was investigated. Pandé and Chopra (*unpublished*) studied the chromosome number and the presence of diplospores in *Physcomitrium* sp., having the haploid number  $n=3$  which apparently is the smallest chromosome count recorded so far in the mosses.

The present contribution deals with the cytology of *Funaria calvescens* Schw., *Bryum cellulare* Hook., *B. ramosum* (Hook.) Mitt., *B. pseudo-pachytheca* C. Müll., *Pohlia elongata* Hedw. and *P. flexuosa* Hook.

MATERIAL AND METHOD

The material of all the species investigated for this communication was collected from Kud which lies at a distance of about 50 miles from Jammu, on Jammu-Srinagar Road, at an altitude of 5,000 ft. above sea-level and Batot another town in the same state situated 15 miles further ahead on the same road at an altitude of about 6,000 ft. above sea-level. Fixation was done in the field in acetic-alcohol (1:3). The observations recorded below are exclusively based on aceto-carmine squash preparations. Capsules in which the operculum is not pigmented were selected for the present study as they yielded the required stages.

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## OBSERVATIONS

1. *Funaria calvescens* Schw., is a member of the family *Funariaceae*. In several capsule squash preparations 14 bivalents were counted at diakinesis and metaphase I in a number of sporocytes (Figs. 1-2). Meiosis is of the normal type.

2. *Bryum cellulare* Hook. belongs to the family *Bryaceae*. In several preparations of the capsule squashes 10 bivalents were counted at diakinesis and metaphase I. Two of the bivalents showed a marked difference in size from the remaining eight, one of them, A, being bigger than the others, while the other one B, is smaller. The rest of the bivalents are, more or less, of intermediate size. The position of the smaller bivalent, with respect to other chromosomes, is variable (compare Figs. 3-4). No laggards were observed during the process of formation of diad and tetrad nuclei.

3. *B. ramosum* (Hook.) Mitt. In a number of sporocytes in several capsule squashes 10 bivalents could be counted at metaphase I (Fig. 5). The meiosis follows the normal course.

4. *B. pseudo-pachytheca* C. Müll. In several acetocarmine capsule squashes 20 bivalents were counted at diakinesis and metaphase I. Two of the bivalents are small, two others are bigger, while the rest are intermediate in size (Figs. 6-7). *B. pseudo-pachytheca* shows two sets of ten bivalents and it can be inferred that this species might have originated from a stock with  $2n = 20$  ( $18 + nM$ ) in the evolution of the species of the genus *Bryum*.

5. *Pohlia elongata* Hedw. belongs to the family *Bryaceae*. In several acetocarmine capsule squashes 11 bivalents could be counted at diakinesis and metaphase I (Figs. 8-9). One of these is conspicuously large as compared to the remaining 10. Meiosis is of the normal type.

6. *P. flexuosa* Hook. 11 bivalents were counted at Metaphase I in a number of sporocytes in aceto-carmine capsule squashes. (Fig. 10).

## DISCUSSION AND CONCLUSION

In their earlier papers Pandé and Chopra (1957a,b) presented in a tabular form the chromosome counts known for the various species of *Funaria* and the Cytological races of *Funaria hygrometrica*. The present communication includes a supplementary list of the chromosome numbers of a few other species. Obviously  $n = 14$ , reported for central European material (Wettstein, 1924) and Californian material of *Funaria hygrometrica* (Vaarama, 1953) is the basic number for the genus *Funaria*.

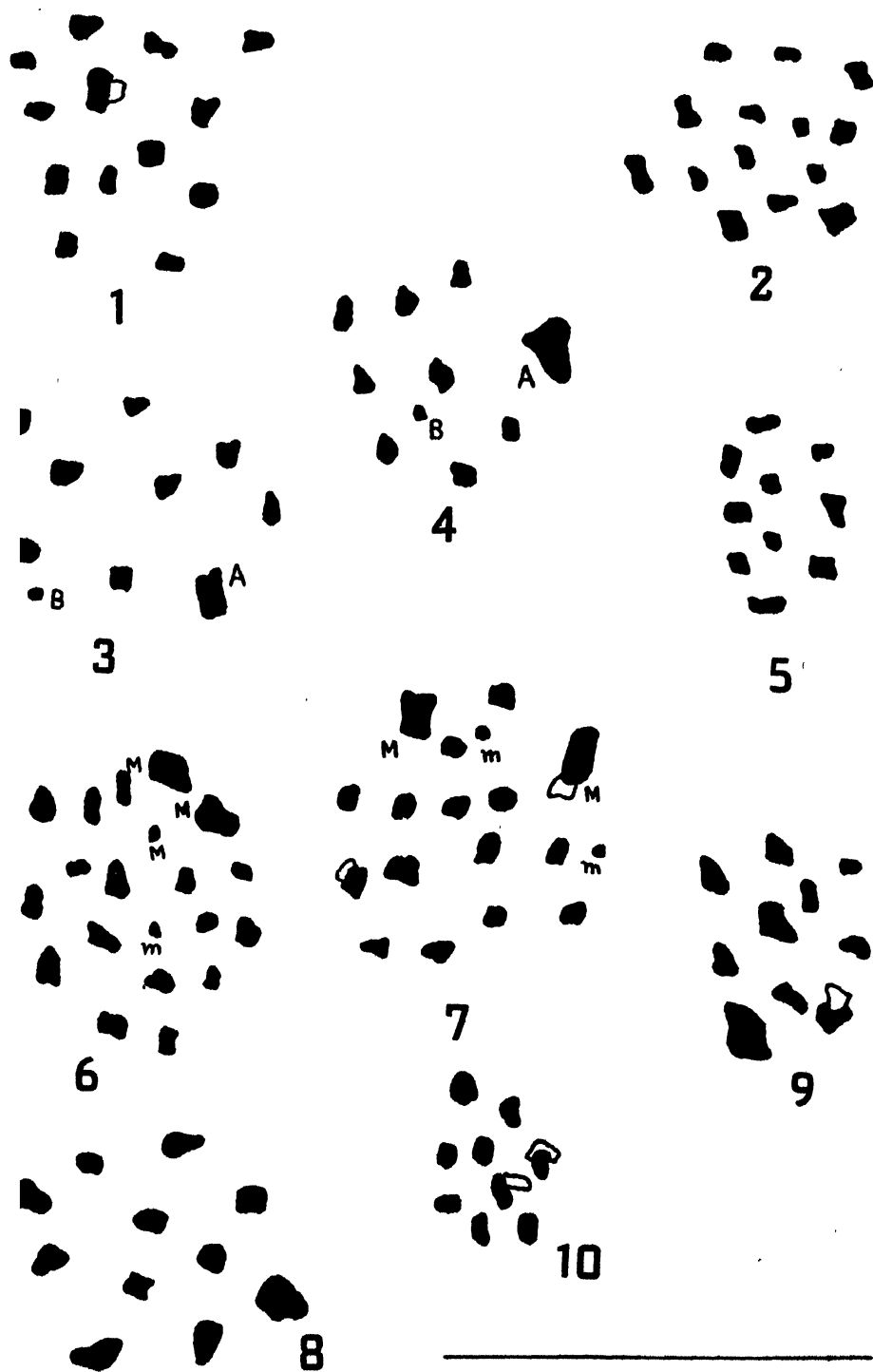
Wettstein (1924) reported  $n = 16$  for *Physcomitrella patens*.

According to Schmidt (1931) the gametophytic chromosome number for the German material of *Physcomitrium pyriforme* is 36, while Pandé and Chopra (1957a) found  $n = 9$  for the same species. In another species of *Physcomitrium* Pandé and Chopra (unpublished) observed two types of sporocytes in the same capsule with  $n = 3$  and  $n = 6$ .

Pandé and Chopra (1957b) reported  $n = 31$  for *Physcomitrellopsis indica*.

It will be evident from the table given below that the basic numbers for *Funaria*, *Physcomitrella*, *Physcomitrium* and *Physcomitrellopsis*, of the family *Funariaceae*, is  $n = 14$ ,  $n = 16$ ,  $n = 3$ ,  $n = 31$  respectively which evolved independently and without any cytological inter-generic relationship whatsoever.

From the chromosome numbers that have so far been given for the species of *Bryum* it can safely be inferred that the basic number for this genus is 10. Out of the five Indian species of *Bryum* that have been worked out (Chopra, 1957a : Pandé and Chopra 1957b), in four of the species, the chromosome number is  $n = 10$  while one shows  $n = 20$ . In *Bryum nitens*, by a treatment of the spores with colchicine, Chopra (1957b) obtained diploid gametophytes experimentally.



For the genus *Pohlia* obviously the basic number is 11.

Table giving the chromosome number of some members of the Families  
Funariaceae and Bryaceae

| Name of the family and the plant          | <i>n</i>       | Author and the year                   |
|---|----------------|---------------------------------------|
| Genus <i>Funaria</i>                      |                |                                       |
| <i>F. muhlenbergii</i> Var. <i>patula</i> | 28             | Steere (1954a).                       |
| * <i>F. calvescens</i>                    | 14             | Pandé and Chopra.                     |
| <i>F. hygrometrica</i>                    | 14             | Wettstein (1924).                     |
| "   | 14             | Vaarama (1953).                       |
| "   | 28             | Vaarama (1950, 1955).                 |
| "   | 28             | Steere (1954a).                       |
| Genus <i>Physcomitrium</i>                |                |                                       |
| <i>P. pyriforme</i>                       | 36             | Schmidt (1931).                       |
| <i>P.</i> "                               | 9              | Pandé and Chopra (1957a).             |
| <i>P.</i> sp.                             | 3 & 6          | Pandé and Chopra ( <i>unpublish</i> ) |
| Genus <i>Physcomitrellopsis</i>           |                |                                       |
| <i>P. indica</i>                          | 31             | Pandé and Chopra (1957b).             |
| Genus <i>Physcomitrella</i>               |                |                                       |
| <i>P. patens</i>                          | 16             | Wettstein (1924)                      |
| Family <i>Bryaceae</i> .                  |                |                                       |
| Genus <i>Bryum</i> .                      |                |                                       |
| <i>B. cyclophyllum</i>                    | 10             | Yano (1956).                          |
| <i>B. nagasakense</i>                     | 10             | "                                     |
| <i>B. pallescens</i>                      | 10             | Yano (1952).                          |
| <i>B. pseudo-alpinum</i>                  | 10             | Yano (1956).                          |
| <i>B. bicolor</i>                         | 10             | Vaarama (1956).                       |
| <i>B. caespiticium</i>                    | 10             | Marchal and Marchal (1911).           |
| <i>B. caespiticium</i>                    | 10             | Yano (1956).                          |
| "   | 20             | Sannomiya (1955).                     |
| <i>B. corrensii</i>                       | 20             | Griesinger (1937).                    |
| "   | 20             | Wettstein and Straub (1942).          |
| "   | 40             | Griesinger (1937).                    |
|   | (Experimental) |                                       |
| <i>B. capillare</i>                       | 10             | Marchal and Marchal (1911).           |
| "   | 20             | " "                                   |
|   | (Experimental) |                                       |
| "   | 10+2-3         | Steere <i>et al.</i> (1954).          |
| <i>B. pseudotriquetrum</i>                | 10             | Heitz (1928).                         |
| "   | 10+1m          | Steere (1954a).                       |

#### EXPLANATION OF TEXT-FIG. 1.

- Figs. 1-2 *Funaria calvescens* Schw.,  
Fig. 1. 14 bivalents at diakinesis.  
Fig. 2. 14 bivalents at metaphase I.
- Figs. 3-4 *Bryum cellulare* Hook.  
Fig. 3. 10 bivalents at diakinesis.  
Fig. 4. 10 bivalents at metaphase.
- Fig. 5. *Bryum ramosum* (Hook.) Mitt.  
Fig. 5. 10 bivalents at metaphase I.
- Figs. 6-7 *Bryum pseudo-pachytheca* C. Müll.  
Figs. 6-7. 20 bivalents at metaphase I, including 2m & 2M pairs.
- Figs. 8-9 *Pohlia elongata* Hedw.  
Fig. 8. 11 bivalents at diakinesis.  
Fig. 9. 11 bivalents at metaphase I.
- Fig. 10. *Pohlia flexuosa* Hook.  
Fig. 10. 11 bivalents at metaphase I.

TABLE—Contd.

| Name of the family and the plant | <i>n</i>       | Author and the year       |
|----------------------------------|----------------|---------------------------|
| * <i>B. cellulare</i>            | 9+1m           | Pandé and Chopra.         |
| * <i>B. ramosum</i>              | 10             | "                         |
| * <i>B. pseudo-pachytheca</i>    | 20             | "                         |
|                                  | (18+Mm)        |                           |
| <i>B. nitens</i>                 | 10             | Pandé and Chopra (1957a). |
|                                  | (9+x or y)     |                           |
| "                                | 20             | Chopra (1957b).           |
|                                  | (Experimental) |                           |
| Genus <i>Pohlia</i>              |                |                           |
| <i>P. cruda</i>                  | 10             | Steere (1954b).           |
| <i>P. scabridens</i>             | 10             | Yano (1956).              |
| <i>P. wahlenbergii</i>           | 11             | Yano (1956).              |
| <i>P. suzuckii</i>               | 20             | "                         |
| <i>P. acuminata</i>              | 11             | "                         |
| <i>P. columbica</i>              | 11             | "                         |
| <i>P. delicatula</i>             | 11             | "                         |
| <i>P. elongata</i>               | 11             | Yano (1953).              |
| * <i>P. "</i>                    | 11             | Pandé and Chopra.         |
| <i>P. revoluta</i>               | 11             | Yano (1956).              |
| <i>P. longicollu</i>             | 22             | Yano (1953).              |
| <i>P. nutans</i>                 | 22             | Vaarama (1956).           |
| <i>P. nutans</i>                 | 22             | Yano (1953, 1956).        |
| <i>P. nutans</i>                 | 21             | Steere (1954b).           |
| * <i>P. flexuosa</i>             | 11             | Pandé and Chopra.         |
| <i>P. longibracteata</i>         | 12             | Steere (1954a).           |

\* based on the present study.

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\*Not seen in original.

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# INCONSTANCY IN CHROMOSOME COMPLEMENTS IN SPECIES OF *MARANTA* AND *CALATHEA*

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## ABSTRACT

1. Nine different species of the family Marantaceae distributed under two genera *Calathea* and *Maranta* have been cytologically studied. The chromosome numbers counted for the different species are :

|                                     |           |
|-------------------------------------|-----------|
| i) <i>Calathea leopardina</i> Regel | $2n = 8$  |
| ii) <i>C. vanenheckei</i> Regel     | $2n = 22$ |
| iii) <i>C. zebrina</i> Lindl.       | $2n = 22$ |
| iv) <i>C. princeps</i> Regel        | $2n = 22$ |
| v) <i>C. ornata</i> Koorn.          | $2n = 26$ |
| vi) <i>Maranta insignis</i> Hort.   | $2n = 26$ |
| vii) <i>M. picta</i>                | $2n = 26$ |
| viii) <i>M. leuconeura</i> E.Morr.  | $2n = 26$ |
| ix) <i>M. keiskei</i> E. Morr.      | $2n = 33$ |

2. Detailed karyotype studies of all these species have been made. On the basis of cytological data, interrelationship between the two genera as well as their present taxonomic status have been discussed. Species with  $2n = 8$  chromosomes have been considered as representing the most primitive condition.

3. Evidence of structural changes of chromosomes playing a rôle in speciation has been gathered specially from their karyotypes.

4. The significance of variation in chromosome number in different individuals of the same species, on the basis of the report of previous as well as the present one, has been discussed in relation to the origin of chromosomal biotypes in nature.

5. Occurrence of structural and numerical alterations in the somatic complements of different species has been noted. In view of ineffective sexual reproduction, their importance in the origin of biotypes and species through vegetative reproduction has been pointed out.

## INTRODUCTION

Species of *Calathea* and *Maranta* propagate strictly through vegetative means. Flowers are either never produced or noted very scarcely in them. Even then, both the genera have got a number of species which are often cultivated specially in the tropical regions because of the ornamentation of their leaves. In addition to these, the importance of *Maranta arundinacea*, the arrowroot yielding plant, is well known. These two genera comprise the major part of the family Marantaceae.

A glance at the available cytological literature on the two genera reveals certain interesting facts (Venkatasubhan, 1946 ; Sato, 1948 ; Simmonds, 1954). A number of species differ from each other with respect to their chromosome number. More interesting is the fact that even in a single species individuals with different chromosome numbers have been found such as *Calathea veitchiana*, *C. lietzei*, *C. zebrina*, *Maranta arundinacea* etc. Chromosome number as low as eight has been reported in *Calathea veitchiana*. In this species, different authors have reported different somatic numbers in the individuals studied by them



(cf. Sato's report of eight chromosomes in *C. veitchiana* and Venkatasubban's report of twenty-six chromosomes in the same, *l.c.*).

The absence of any sexual reproduction in two such successful genera obviously makes them interesting materials for a study of the mechanism of speciation. Unfortunately no serious attempts have been made to study this aspect so far. In plants reproducing through asexual means certain peculiarities in the chromosome behaviour are noted which are correlated to the origin of species in such plants to a remarkable degree. It has been thought worthwhile to find out whether in the members of the family *Marantaceae* too the same type of behaviour is present. Moreover, the literature shows that a number of species growing in India still remain uninvestigated. Because of this, and also taking into account the fact that these two genera provide ample scope for cytological studies, the present investigation has been undertaken.

Fortunately, as the text would reveal, significant peculiarities in the chromosome behaviour of these species have been obtained which throw considerable light to an understanding of the method of speciation. From the records so far gathered including those of the previous authors, an attempt has been made here to suggest the mechanism of speciation operating in them and to work out the systematic status and the affinities of the two genera.

## MATERIALS AND METHODS

### A. Materials

The following species of plants were the source of materials for the purpose of the present investigation :

1. *Calathea leopardina* Regel
2. *C. princeps* Regel
3. *C. vandenheckei* Regel
4. *C. zebrina* Lindl.
5. *C. ornata* Koern.
6. *Maranta insignis* Hort.
7. *M. leuconeura* E. Morr.
8. *M. picta*
9. *M. kegeljani* E. Morr.

Most of the species of the genera *Calathea* and *Maranta* are inhabitants of tropical America and are generally found in moist or swampy regions. But several species are cultivated in India as ornamental garden plants and also for commercial purposes. Ornamentation of their foliage is very diverse including stripes, spots, and various shades of colour on the upper and lower surfaces.

The materials were collected from a local nursery of Calcutta and were grown in a moist shady nursery of the University compound. For root-tips, rhizomes were placed in suitable earthenware pots in a mixture of sand and soil. Another set of plants was grown in garden plots for the initiation of flowers.

### B. Methods

Study of somatic chromosomes presented much difficulty. Various metallic and non-metallic fixatives with varying proportions were used. Different types of pre-treatment chemicals were tried to get good results. Trials were given in Aesculin, Para-dichlorobenzene, Coumarin, and Newcomer's fluid (Newcomer, 1953). It was found that a mixture of Newcomer's fluid and Para-dichlorobenzene (aqueous) in the proportion of 1 : 1 yielded best results. But the main difficulty with the somatic chromosomes is their very weak stainability and to overcome this, a modified technique was applied. This was as follows :

Newcomer's fluid (Iso-propyl alcohol : Propionic acid : Ether : Acetone : Dioxan :: 6 : 3 : 1 : 1 : 1) and aqueous solution of Para-dichlorobenzene were mixed in the proportion of 1 : 1 just before treatment. Healthy root-tips were next treated in this mixture for  $1\frac{1}{2}$  hours at  $10^{\circ}\text{C}$ . The materials were then hydrolysed and stained in a mixture of 2 per cent Aceto-orcein and N/HCl (9 : 1) by heating over a flame for a few seconds. Stained root-tips were next transferred to 1 per cent iron alum solution (aqueous) and heated for a few more seconds. The materials were then immediately placed in 1 per cent Aceto-orcein solution and squashed. Slides were sealed properly. The peak period of mitotic division was found to occur between 10 A.M. to 12 noon. Paraffin sections,  $14\mu$  thick, were also cut and stained following Newton's crystal violet technique.

The figures were drawn at a table magnification  $\times 29,00$  using a Zeiss compensating eye-piece of  $\times 20$  and a 1.3 apochromatic objective.

In the drawings, chromosomes with secondary constrictions have been drawn in outline.

### OBSERVATIONS

All the different species are perennial herbs with rhizomatous stems. Leaves are borne in two rows, each leaf differentiated into an open sheath, stalk and a blade.

Nine species distributed under the two genera, *Calathea* and *Maranta*, have been cytologically examined in the present case. The somatic chromosome number in them has been found to range from as low as  $2n : 8$  to as high as  $2n = 33$ . Nuclei with varying number of chromosomes in their complements, involving both structural and numerical alterations have been observed in all of them. That somatic number which occurs in the highest frequency, is taken as the normal number for the species.

### GENUS—*Calathea*

Five species were investigated. Of these, three are characterized by twenty-two, one by twenty-six and the remaining one by having only eight chromosomes in their somatic complements respectively.

The chromosomes show no marked size difference but a slow gradation in size is found within the complement of the different species. The number of chromosomes with secondary constrictions varies from two to eight. Chromosomes with supernumerary constrictions are found in two species. Size of the chromosomes ranges from  $1.1\mu$  to  $3.4\mu$  and on its basis three general groups can be recognized, viz., comparatively long, medium and short. Primary constrictions are mostly median to submedian in position.

All the different species of this genus reveal a gross similarity as regards the morphology of the chromosomes. So it will be convenient to describe the general chromosome types first. Their finer details will be described under the karyotype analysis for each species. Following are the main types:—

Type A—Represents medium sized chromosomes each having three constrictions, one primary and two secondary, of which one is nearly median in position and the other two in submedian positions at the two opposite ends.

Type A<sub>1</sub>—Medium sized chromosomes each with three constrictions, one primary and two secondary, of which one is nearly submedian in position, another located at the middle of the shorter arm and the third nearly submedian in position at the distal end of the longer arm,

- Type B**—Medium sized chromosomes (comparatively long in one case) each with two constrictions, primary and secondary, one nearly median and the other nearly submedian, placed very near the former.
- Type C**—Comparatively long chromosomes each possessing two constrictions, primary and secondary, one nearly median and the other nearly subterminal at the distal end of the comparatively longer arm.
- Type D**—Medium sized chromosomes each with two constrictions, primary and secondary, both in submedian positions at the two distal ends. It differs from E in having the position of chromosome arm between the two constrictions much longer in size than the other two distal parts.
- Type E**—Medium sized chromosomes each with two constrictions, primary and secondary, both located in nearly submedian positions at the opposite ends of the chromosomes.
- Type F**—Medium sized (comparatively long in some cases) chromosomes with nearly submedian to submedian primary constrictions.
- Type G**—Short (comparatively medium sized in a few pairs) chromosomes with nearly median to median primary constrictions.
- Type H**—Short to very short chromosomes with median primary constrictions.

1. *C. leopardina* Regel ( $2n = 8 - 2M^* + 4M + 2S$ )

Herbaceous perennials with small root-stock and a few small leaves. Petioles short, leaf-surface with white patches along the mid-rib.

The somatic chromosome number in the normal plate is found to be  $2n = 8$  (Fig. 1). Size difference is not marked and two groups can be recognised.

- i) Three pairs of medium sized chromosomes, and
- ii) One pair of short chromosomes.

Of these, only one pair of chromosomes is found to bear secondary constrictions. The size range varies between  $1.6\mu$  to  $2.8\mu$ .

Detailed karyotype analysis is shown in the following table (Table I, Fig. 2) :—

TABLE I

*Karyotype analysis in C. leopardina*

| Type | Number  | Special features                         |
|------|---------|--|
| D    | 1 pair  | Comparatively longer than other D types. |
| F    | 2 pairs | One pair slightly longer than the other. |
| G    | 1 pair  | Common G type.                           |

In addition to the normal karyotype, a variation plate with twelve chromosomes is also on record (Fig. 3). In the somatic prophase of the normal plate two chromosomes are found to be attached with the nucleolus (Fig. 4).

2. *C. princeps* Regel ( $2n = 22 = 2L^* + 2M^* + 10M + 8S$ )

Leaves long-petioled, long and narrow, upper surface dark green and glossy ; lower surface pink.

Twenty-two chromosomes are present in the complements of normal somatic cells (Fig. 5). Size difference is comparatively marked, and on its basis, the chromosomes can be divided into the following general groups :—

- i) One pair of comparatively long chromosomes,
- ii) Six pairs of medium sized chromosomes, and
- iii) Four pairs of short to very short chromosomes.

Of these, four chromosomes bear secondary constrictions. The size ranges from  $1.2\mu$  to  $3.1\mu$ .

Table II shows the detailed analysis of the karyotype (Fig. 6).

TABLE II

*Karyotype analysis in C. princeps*

| Type | Number  | Special features                                   |
|------|---------|--|
| C    | 1 pair  | Normal C type.                                     |
| F    | 1 "     | Longer than other F type.                          |
| E    | 1 "     | Common E type.                                     |
| G    | 6 pairs | Two pairs comparatively longer than normal G type. |
| H    | 2 "     | Common H type.                                     |

In addition to the normal karyotype, a cell with abnormal number of chromosomes ( $2n = 20$ ) is also on record (Fig. 7).

### 3. *C. vandenheckei* Regel ( $2n = 22 = 2L^s + 8M + 12S$ )

Leaves large, ovate, long-petioled, upper surface dark green and glossy, lower surface pink. Pale yellow patches along the mid-rib conspicuous.

The normal somatic cells of the species contain  $2n = 22$  chromosomes (Fig. 8). Beside one pair of comparatively long chromosomes, the rest are medium sized to short forming a graded series. Size difference makes the chromosomes divisible into three groups.

- i) One pair of comparatively long chromosomes,
- ii) Four pairs of medium sized chromosomes, and
- iii) Six pairs of short to very short chromosomes.

Of these chromosomes, only one pair is seen to bear secondary constrictions. The size ranges from  $1.2\mu$  to  $3.4\mu$ . Detailed study of the chromosome morphology is revealed from the following table (Table III, Fig. 9) :—

TABLE III

*Karyotype study in C. vandenheckei*

| Type | Number  | Special features           |
|------|---------|----------------------------|
| B    | 1 pair  | Longer than other B types. |
| F    | 3 pairs | Common F type.             |
| G    | 4 "     | " G "                      |
| H    | 3 "     | " H "                      |

Beside the normal karyotype, a variation plate with  $2n = 24$  chromosomes is on record (Fig. 10). Late separation of chromosomes forming an apparent somatic bridge (Fig. 11) and lagging chromosomes are also observed (Fig. 11a).

### 4. *C. zebrina* Lindl. ( $2n = 22 = 2M'' + 4M' + 4M + 12S$ )

Leaves many, ovate-lanceolate, pale green with light black stripes diverging from the mid-rib.

$2n = 22$  chromosomes are found in cells with normal somatic complements (Fig. 12). Size difference is not marked and two general groups can be recognized.

- i) Five pairs of medium-sized chromosomes, and
- ii) Six pairs of short to very short chromosomes.

Three pairs of chromosomes are found to bear secondary constrictions, one pair of which possesses supernumerary constrictions. The size range varies between  $1.2\mu$  to  $2.7\mu$ . Detailed karyotype analysis is shown in Table IV (Fig. 13).

TABLE IV

*Analysis of Karyotype in C. zobrina*

| Type | Number  | Special features            |
|------|---------|-----------------------------|
| A    | 1 pair  | Normal A type.              |
| D    | 1 pair  | Shorter than common D type. |
| E    | 1 pair  | " " E "                     |
| F    | 2 pairs | Common F type               |
| G    | 2 pairs | " G "                       |
| H    | 4 pairs | " H "                       |

In addition to the normal karyotype, several variation plates, involving both structural and numerical changes in chromosomes are on record. Numbers both lower and higher than the normal  $2n = 22$  plates are found viz.,  $2n = 20, 23, 25, 26$  and  $30$  (Figs. 14–18). Nuclei maintaining the normal number show structural alterations in some cases (Fig. 18a). Somatic irregularities namely, lagging and late separation of chromosomes are also observed.

#### 5. *C. ornata* Koern. ( $2n = 26 = 2M^{ss} + 6M^s + 18S$ )

Leaves numerous, large, pink beneath, upper surface dark green with fine white stripes.

Normal somatic complement is found to contain twenty-six chromosomes (Fig. 19). Size difference is not so marked, and on its basis following two general groups can be recognized:—

- i) Four pairs of medium sized chromosomes, and
- ii) Nine pairs of short to very short chromosomes.

Of these, eight chromosomes bear secondary constrictions including one pair with supernumerary constrictions. The size ranges from  $1.1\mu$  to  $2.8\mu$ . Detailed morphology of the chromosomes is revealed in Table V (Fig. 20).

TABLE V

*Karyotype analysis in C. ornata*

| Type           | Number  | Special features   |
|----------------|---------|--|
| A <sub>1</sub> | 1 pair  | Normal A <sub>1</sub> type.                                  |
| B              | 1 "     | Shorter than other B types.                                  |
| E              | 2 pairs | One constriction in each of the chromosomes much pronounced. |
| G              | 7 "     | A few pairs shorter than general G type.                     |
| H              | 2 "     | Common H type.   |

Variation plates with different chromosome numbers, such as  $2n = 19, 24$  and  $30$  are also on record (Figs. 21–23). Somatic irregularities such as, non-disjunction and lagging are observed in some cases.

GENUS—*Maranta*

Four species belonging to this genus were studied. Of these, three are characterized by twenty-six and the other one by thirty-three chromosomes. Besides these, several variation plates are also on record.

The chromosomes show no well marked size difference within the complement, but they form a graded series. Secondary constrictions are found to occur in four to nine chromosomes. Two species reveal chromosomes with supernumerary constrictions. Size of the chromosomes ranges from  $1.2\mu$  to  $4.1\mu$ . They are mostly medium to short in size except in *M. kegeljani*, in which type  $A_1$  is comparatively longer than in the rest of the species.

In the detailed karyotypes of the different species, the general chromosome types are described first on a comparative basis and the minor differences shown separately. The principal types are:—

Type A—Medium sized to comparatively long chromosomes each with three constrictions, one primary and two secondary, of which one is nearly median and the other two in submedian positions at the opposite ends.

Type  $A_1$ —Long chromosome having three constrictions, one primary and the other two secondary. One constriction is median and the other two are placed in submedian positions at the two arms. This type is much longer than the A type.

Type B—Medium sized chromosomes each with two constrictions, primary and secondary, one nearly median and the other nearly subterminal at the distal end of the comparatively longer arm.

Type C—Medium sized chromosomes each with two constrictions, primary and secondary, both located in nearly submedian positions at the opposite ends of the chromosomes.

Type D—Medium sized chromosomes with nearly submedian to submedian primary constrictions.

Type E—Short chromosomes with nearly median to median primary constrictions.

Type F—Very short chromosomes with median primary constrictions.

6. *M. insignis* Hort. ( $2n = 26 = 4M^s + 4M + 18S$ )

Leaves small, narrow, shortly petioled with undulating margin.

The somatic chromosome number in the normal plate is found to be  $2n = 26$  (Fig. 24). Size difference is not marked and two groups can be recognised.

i) Four pairs of medium sized chromosomes.

ii) Nine pairs of short to very short chromosomes.

Four chromosomes are found to bear secondary constrictions. The size range varies between  $1.2\mu$  and  $2.1\mu$ . Detailed karyotype analysis is shown in the following table (Table VI, Fig. 25):—

TABLE VI

*Analysis of karyotype in M. insignis*

| Type | Number  | Special features                     |
|------|---------|--------------------------------------|
| B    | 1 pair  | Common B type.                       |
| C    | 1 "     | Slightly shorter than common C type. |
| D    | 2 pairs | Common D type                        |
| E    | 7 "     | " E "                                |
| F    | 2 "     | " F "                                |

In addition to the normal karyotypes, a variation plate with  $2n = 22$  chromosomes is on record (Fig. 26).

7. *M. leuconeura* E. Morr. ( $2n = 26 = 2L^s + 2M^{ss} + 2M + 20S$ )

Leaves small, ovate, upper surface dark green with light patches.

Twentysix chromosomes are found in cells with normal somatic complements (Fig. 27). Size difference is present and on its basis, the following general groups can be recognised :

- i) One pair of comparatively long chromosomes.
- ii) Two pairs of medium sized chromosomes.
- iii) Ten pairs of short to very short chromosomes.

Two pairs of chromosomes bear secondary constrictions including one pair with supernumerary constrictions. The size difference varies from  $1.2\mu$  to  $3.1\mu$ . Table VII shows the detailed analysis of the karyotype (Fig. 28).

TABLE VII

*Karyotype analysis in M. leuconeura*

| Type | Number  | Special features   |
|------|---------|--|
| A    | 1 pair  | Comparatively longer in size and the arm at one end is also slightly longer than the general A type. |
| C    | 1 „     | Common C type.   |
| D    | 1 „     | „ D „  |
| E    | 7 pairs | Common E type forming a graded series.   |
| F    | 3 „     | Common F type.   |

Some nuclei with  $2n = 26$  chromosomes show structural alterations in their complements (Fig. 29). Numerically altered nuclei ( $2n = 28$ ) are also observed in a few cases (Fig. 30).

8. *M. picta* ( $2n = 26 = 6M^s + 6M + 14S$ )

Plants small, leaves ovate, very thin, pale green with beautiful dark spots on the upper surface.

The normal somatic complement is found to contain twentysix chromosomes (Fig. 31). Size difference is not marked and two general groups can be recognised.

- i) Six pairs of medium sized chromosomes, and
- ii) Seven pairs of short to very short chromosomes.

Of these, six chromosomes bear secondary constrictions. The size ranges between  $1.2\mu$  and  $2.5\mu$ . Detailed karyotype is revealed in Table VIII (Fig. 32).

TABLE VIII

*Karyotype analysis in M. picta*

| Type | Number  | Special features  |
|------|---------|---|
| B    | 1 pair  | Common B type   |
| C    | 2 pairs | One constriction in each of a pair of chromosomes is much shorter than the other. |
| D    | 3 „     | Common D type   |
| E    | 4 „     | „ E „   |
| F    | 3 „     | „ F „   |

One variation plate with  $2n = 33$  chromosomes is also noticed (Fig. 33).

9. *M. kegeljani* E. Morr. ( $2n = 33 = 1L_1^s + 2L^{ss} + 2L + 6M + 8M + 16S$ )

Leaves numerous, oblong, long petioled, yellowish green, margins parallel. Upper surface very glossy.

Thirty three chromosomes are found in the cells with normal somatic complements (Fig. 34). Size difference is comparatively marked and on its basis the following groups can be recognized :—

- i) One long chromosome, the longest in the set,
- ii) One pair of comparatively long chromosomes,
- iii) Five pairs of medium sized chromosomes, and
- iv) Ten pairs of short to very short chromosomes.

Altogether nine chromosomes are found to bear secondary constrictions. of these, three bear supernumerary constrictions. The size difference varies from,  $1.2\mu$  to  $4.1\mu$ . Detailed karyotype is described in the following table (Table IX Fig. 35) :—

TABLE IX

*Analysis of karyotype in M. kegeljani*

| Type  | Number   | Special features   |
|-------|----------|--|
| $A_1$ | One only | Longest chromosome with supernum-<br>erary constriction. |
| A     | 1 pair   | Common A type  |
| B     | 1 "      | " B "  |
| C     | 2 pairs  | One constriction in each of a pair much<br>pronounced.   |
| D     | 2 "      | Common D type  |
| E     | 6 "      | " E "  |
| F     | 4 "      | " F "  |

In addition to the normal karyotype, a large number of somatic nuclei with abnormal chromosome complements, involving both structural and numerical alterations, are also on record. Variations in chromosome number found are  $2n = 20, 26, 30, 36, 41, 42, 44$  and  $47$  (Figs. 36–43). Lagging chromosomes at anaphase are also recorded in some cases (Fig. 44). Some  $2n = 33$  complements exhibit structural alterations of chromosomes (Fig. 45).

## DISCUSSION

### 1. Variation in chromosome number and the origin of chromosomal biotypes.

The two genera *Calathea* and *Maranta*, included under the tribes Phrynieceae and Marantaceae respectively, are considered by taxonomists as very much related to each other (Engler and Prantl, 1930 ; Hutchinson, 1934). A number of species already included under the genus *Maranta* have later been transferred to the genus *Calathea*.

Cytological literature reveals that so far as the genus *Calathea* is concerned, multiples of four, eleven, twelve and thirteen chromosomes have been found in the complements of the different species (Venkatasubban, 1946 ; Sato, 1948). Eight chromosomes have only been reported in *C. veitchiana* by Sato (*l.c.*) and in the present report, *C. leopardina* has also been shown to possess  $2n = 8$  chromosomes. Different species are also on record with  $2n = 22, 24$  and  $52$  chromosomes respectively (vide Table X). Further, in each of the species of *C. lietzei* and



*C. zebrina*, plants have been found with  $2n = 24$  and  $26$  chromosomes by Venkatasubban (l.c.). Individuals of *C. zebrina* reported in the present paper, on the other hand, show  $2n = 22$  chromosomes indicating the presence of a large number of chromosomal biotypes in these species. Reports of chromosome numbers in *C. leopardina* ( $2n = 8$ ), *C. vandenheckei* ( $2n = 22$ ), *C. ornata* ( $2n = 26$ ) and *C. princeps* ( $2n = 22$ ) have been made for the first time in this paper.

TABLE X

*Previous and present records of chromosome number in Calathea and Maranta*

| Species                         | Previous record |                       | Present record<br>( $2n$ No.) |
|---------------------------------|-----------------|-----------------------|-------------------------------|
|                                 | ( $2n$ No.)     | Author                |                               |
| I. <i>Calathea</i>              | 8               | {Sato (1948)          | —                             |
| <i>veitchiana</i>               | 26              | {Venkatasubban (1946) | —                             |
| <i>C. leopardina</i>            | —               | —                     | 8                             |
| <i>C. mediopicta</i>            | 22              | Venkatasubban (1946)  | —                             |
| <i>C. insignis</i>              | 22              | Sato (1948)           | —                             |
| <i>C. vandenheckei</i>          | —               | —                     | 22                            |
| <i>C. princeps</i>              | —               | —                     | 22                            |
| <i>C. zebrina</i>               | 24, 26          | Venkatasubban (1946)  | 22                            |
| <i>C. grandiflora</i>           | 24              | —                     | —                             |
| <i>C. ornata</i>                | —               | —                     | 26                            |
| <i>C. lietzei</i>               | 24, 26          | Venkatasubban (1946)  | —                             |
| <i>C. lindeniana</i>            | 26              | —                     | —                             |
| <i>C. makoyana</i>              | 26              | —                     | —                             |
| <i>C. roseo-picta</i>           | 26              | —                     | —                             |
| <i>C. taeniosa</i>              | 52              | Sato (1948)           | —                             |
| II. <i>Maranta nitida-picta</i> | 8               | Venkatasubban (1946)  | —                             |
| <i>M. arundinacea</i>           | —               | —                     | —                             |
| <i>v. variegatum</i>            | 18              | Sato (1948)           | —                             |
| W. I. Arrowroot                 | 48              | Simmonds (1954)       | —                             |
| <i>M. bicolor</i>               | 24              | Venkatasubban (1946)  | —                             |
| <i>M. tigrina</i>               | 24              | —                     | —                             |
| <i>M. insignis</i>              | —               | —                     | 26                            |
| <i>M. picta</i>                 | —               | —                     | 26                            |
| <i>M. leuconeura</i>            | —               | —                     | 26                            |
| <i>v. massangeana</i>           | 26              | Venkatasubban (1946)  | —                             |
| <i>M. nitida</i>                | 26              | —                     | —                             |
| <i>M. striata</i>               | 26              | Sato (1948)           | —                             |
| <i>M. kegeljani</i>             | —               | —                     | 33                            |

The occurrence of different chromosome numbers in this genus does not necessarily indicate that they belong to different complexes. This is mainly borne out by the fact that even in the same species, different chromosomal biotypes such as,  $2n = 22$ ,  $24$  and  $26$  found in different individuals, as noted in *C. lietzei* and *C. zebrina*, are in existence. This obviously implies that the different chromosome numbers noted in species of *Calathea* are all representatives of a common assemblage, representing in all probability a single evolutionary line. From the same stock, different species have evolved and their specific status has gradually been attained through continuous accumulation of new variations.

Evidently in this line of evolution, individuals of species with  $2n = 8$  chromosomes approach more towards the ancestral forms. That species with  $2n = 8$  chromosomes, such as, *C. veitchiana*, belong to the same evolutionary line, is also indicated by the occurrence of individuals with  $2n = 26$  chromosomes in the same species (Venkatasubban, 1946). At the present state of our knowledge, therefore one can safely assume that *C. veitchiana* and *C. leopardina* represent more of the

ancestral condition than the other species. That all these species belong to the same assemblage, is also evidenced by a gross resemblance in their general morphology of the chromosomes.

Similar to the genus *Calathea*, in species of *Maranta* too, different chromosome numbers have been reported in different species. The previous literature shows that chromosome numbers such as  $2n = 8, 24$  and  $26$  are present in different species (Venkatasubban, 1946; Sato, 1948; Simmonds, 1954). In *M. arundinacea*, both  $2n = 18$  and  $48$  chromosomes have been reported in different varieties. Of the species reported in the present paper, three are characterized by  $2n = 26$  chromosomes and one with  $2n = 33$  chromosomes. The occurrence of such widely different chromosome numbers even in the same genus is quite significant. It has already been emphasized that even in the same species as *M. arundinacea*, individuals are in existence with widely differing chromosome numbers. One of them is designated as a distinct variety. Existence of individuals in the same species with different chromosome numbers finds parallel in species of *Calathea* though however in the latter genus such an occurrence has been noticed in a number of species. It is not unlikely that in other species of *Maranta* too a thorough search may reveal such chromosomal biotypes. In that case, as in *Calathea*, here also all the species may represent members of side branches of a single line of evolution with possibly *M. nitida-picta* with  $2n = 8$  chromosomes approaching more towards the ancestral forms. The present investigation provides support to such an assumption. Of the four species investigated here, three, namely, *M. insignis*, *M. leuco-neura* and *M. picta* contain  $2n = 26$  chromosomes and one, that is *M. kegeljani*,  $2n = 33$  chromosomes. Though the species differ with respect to minor details of karyotype even then, a gross resemblance in general morphology of chromosomes is noticed between them. The species of *Maranta* so far investigated represent in all probability a common line like the species of *Calathea*.

In this connection it is worth noting that investigations on other species of *Calathea* and *Maranta* by Venkatasubban (*l.c.*) led him to consider that at least two different evolutionary lines are running within the genera, one starting with the basic set of  $n = 4$  and other with  $n = 6$  chromosomes. So far as the present observation shows there is no reason to assume two different evolutionary lines within the two genera. *Calathea leopardina*, of the present report, with  $2n = 8$  chromosomes does not show much difference in chromosome size from that of the other species. Further, within the same species of *Calathea veitchiana* both  $2n = 8$  and  $2n = 26$  chromosomes have been reported in different individuals (Venkatasubban, Sato, *l.c.*). Taking all these factors into account it seems better to consider all of them as representatives of a single line of evolution.

## 2. Structural difference of chromosomes between different species of *Maranta* and *Calathea*.

The problem naturally arises as to how such chromosomal biotypes originate. In this connection the means of propagation of the species seems worthy of note. They are all propagated through vegetative means and flowering and seed-setting are very rare. Even when flowering is noted, so far as the author is aware, setting of viable seeds is never observed. So the origin of such chromosomal biotypes through sexual reproduction is absolutely impossible.

It is significant to note that all the species of *Maranta* and *Calathea* show a characteristic peculiarity in the somatic cells, that is the presence of variable chromosome complement in the same tissue (vide Table XI). Such valuable complements involve both numerical and structural alteration of the normal complement. Such behaviour has been encountered in a large majority of the vegetatively reproducing plants so far cytologically studied here. On the basis of considerable evidence this behaviour has been claimed to play a significant rôle in speciation through the participation of the altered nuclei in the formation of

daughter shoots (Sharma and Das, 1954; Mookerjee, 1955; Sharma, 1956; Bhattacharyya, 1957).

In *Maranta* and *Calathea* too, in the absence of sexual method of reproduction, this behaviour probably accounts for their speciation through vegetative means as noted in many other members of higher plant groups reproducing through asexual means.

TABLE XI

*Difference in chromosome morphology, variations and length of chromatin matter in Calathea and Maranta*

| Species                         | Normal somatic number (2n) | Size difference in diploid complement (2n)                                    | Variation in somatic number (2n)        | Total amount of chromatin matter in haploid complement (accounted in length) |
|---------------------------------|----------------------------|---|---|--|
| 1. <i>Calathea leopardina</i> . | 8                          | **2M <sup>s</sup> + 4M + 2S   | 12                                      | 9.3μ   |
| 2. <i>C. vanenheckei</i>        | 22                         | 2L <sup>s</sup> + 8M + 12S  | 24                                      | 19.5μ  |
| 3. <i>C. princeps</i>           | 22                         | 2L <sup>s</sup> + 2M <sup>s</sup> + 10M + 8S                                  | 20                                      | 22.2μ  |
| 4. <i>C. zebrina</i>            | 22                         | 2M <sup>ss</sup> + 4M <sup>s</sup> + 4M + 12S                                 | 20, 22*, 23, 25, 26 & 30                | 18.0μ  |
| 5. <i>C. ornata</i>             | 26                         | 2M <sup>ss</sup> + 6M <sup>s</sup> + 18S                                      | 19, 24 & 30                             | 21.7μ  |
| 6. <i>Maranta insignis</i> .    | 26                         | 4M <sup>s</sup> + 4M + 18S  | 22                                      | 18.6μ  |
| 7. <i>M. picta</i>              | 26                         | 6M <sup>s</sup> + 6M + 14S  | 33                                      | 22.4μ  |
| 8. <i>M. leuconeura</i>         | 26                         | 2L <sup>ss</sup> + 2M <sup>s</sup> + 2M + 20S                                 | 26* & 28                                | 20.7μ  |
| 9. <i>M. kegeljani</i>          | 33                         | 1L <sub>1</sub> <sup>ss</sup> + 2L <sup>ss</sup> + 6M <sup>s</sup> + 4M + 20S | 20, 26, 30, 33,*<br>36, 41, 42, 44 & 47 | 20.5μ<br>(½ of the 2n number)  |

\* Normal number but with structural alterations.

\*\* L<sub>1</sub>—Very long chromosome.

L — Long chromosome

M — Medium sized chromosome

S — Short chromosome

L<sup>s</sup>, M<sup>s</sup> — Long or medium sized chromosome with secondary constriction.

L<sub>1</sub><sup>ss</sup>, L<sup>ss</sup>, M<sup>ss</sup> — Very long, long or medium sized chromosome with supernumerary constrictions.

Now the problem arises regarding the way through which such abnormalities originate in the tissue. The only means that can be considered as responsible for their origin is mitotic irregularities. Non-disjunction, lagging etc., (as mentioned in the text) can possibly account for their origin. Though in case of other species, somatic reduction has been found to occur accounting for the origin of such abnormal number (Sharma, 1956), no evidence of such somatic reduction has been obtained in the present observation. However, unless a thorough search in other genera is made, it is not possible to state whether somatic reduction has played a prominent rôle in the origin of such abnormal numbers. Venkatasubban (1946), however, just suggested that so far as the number 2n = 22 in *C. mediopicta* is concerned, it may be derived from the number 2n = 24 by the fusion of four chromosomes in pairs as suggested by Sato (1939) in certain monocotyledonous members. But if the karyotypes of the different species of *Maranta* and *Calathea* shown in the present paper and the chromosome drawings given in the previous records are taken into consideration, no such indication of chromosome fusion seems to be evident. Further, in *Calathea veitchiana* both 2n = 8 and 2n = 26 chromosomes have been reported. Besides, if one is to assume chromosome fusion, then one is also to infer that eight chromosomes of *C. veitchiana* might have been

derived from twenty-six chromosomes of the same species through continuous fusion of chromosome ends. But such an assumption seems to be highly speculative in view of the absence of any experimental evidence confirmatory to this statement.

It has already been pointed out that though the different species of the genera *Maranta* and *Calathea* show a good deal of resemblances in chromosome morphology and total amount of chromatin matter, minute karyotypic differences are there delimiting one species from another. Each and every species has got a distinct karyotype of its own. This obviously indicates the rôle of structural alteration of chromosomes in the evolution of the different species of these genera, as noted in a number of other plant species too (Bergner, Satina and Blakeslee, 1933; Bhaduri, 1944; Wilkinson, 1944; Goodspeed, 1945; Babcock, 1947; Chakravorti, 1951; Sharma and Ghosh, 1954; Sharma and De, 1956; Sharma and Bhattacharjee, 1957; and Stebbins, 1951). Such structural alteration seems to have affected mainly the chromosomes with more than one constrictions and such species differ with respect to the number of secondary constrictions present in them. The existence of chromosomes with supernumerary constrictions also indicates the evidence of structural changes undergone by these species.

It may be noted that in *Calathea vandenheckei*, only two chromosomes bearing secondary constrictions or satellites have been observed. The presence of such a low number of secondary constrictions in a species having a chromosome number as high as twenty-two seems to be interesting especially in view of the fact that in species with eight chromosomes also two secondary constrictions are present. It is not unlikely that structural changes involving amphiplasty have resulted into the loss of secondary constrictions in *C. vandenheckei*.

### 3. *Interrelationship between the genera Calathea and Maranta.*

This discussion will remain incomplete if the interrelationship between *Calathea* and *Maranta*, as far as can be traced from cytological data, is not pointed out. So far as the observations reveal, their members show a good deal of similarity in a number of respects. The chromosome number and its ranges are practically the same in both. The morphology of the chromosomes too indicates close resemblances. The complements of both are characterised by mostly medium to short chromosomes having not much of size difference in the complement. The primary constrictions mostly range from median to submedian in position. The range in the number of secondary constrictions too does not show much difference between these two genera. In view of these facts, it may safely be considered that so far as the cytological data are concerned they give ample evidence for their close relationship. Their inclusion within the same family Marantaceae by the taxonomists is fully justified (Engler and Prantl, Hutchinson *l.c.*). It has already been mentioned that a number of species of *Maranta* have, according to the recent nomenclature, been transferred to the genus *Calathea* (vide Index Kewensis upto 1950). All these facts indicate their affinities. However, the taxonomists have included these under two different tribes under the same family Marantaceae. In the absence of any data from other aspects of study it is not possible to state whether their inclusion under different tribes mainly on morphological grounds is justified or not. This much is certain that their inclusion within the same family finds full confirmation from their cytological data.

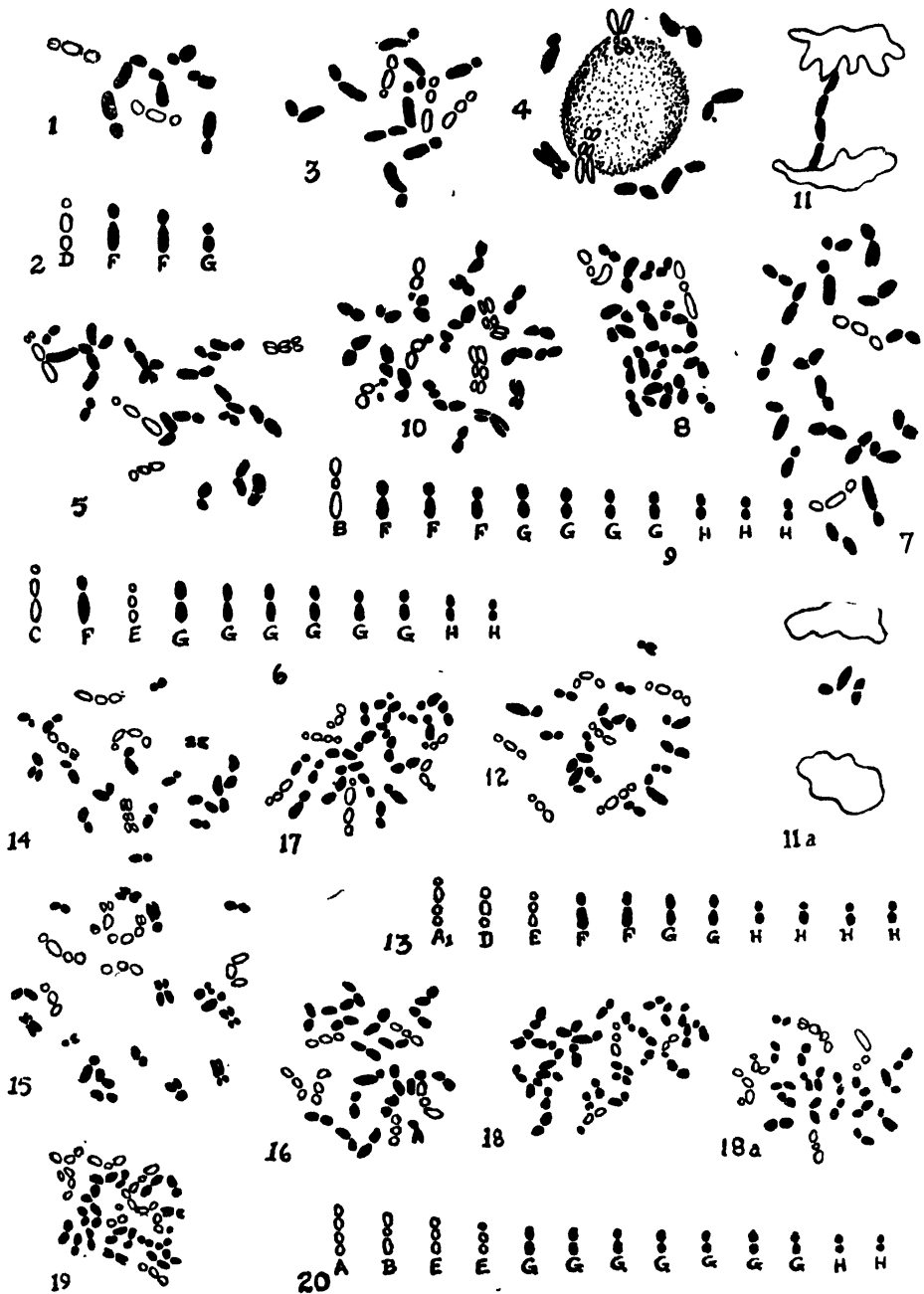
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\* Not consulted in original.



TEXT-FIG. 1.

Figs. 1-3.—*Calathea leopardina*. Normal somatic metaphase ( $2n = 8$ ), idiogram and variation metaphase with 12 chromosomes.

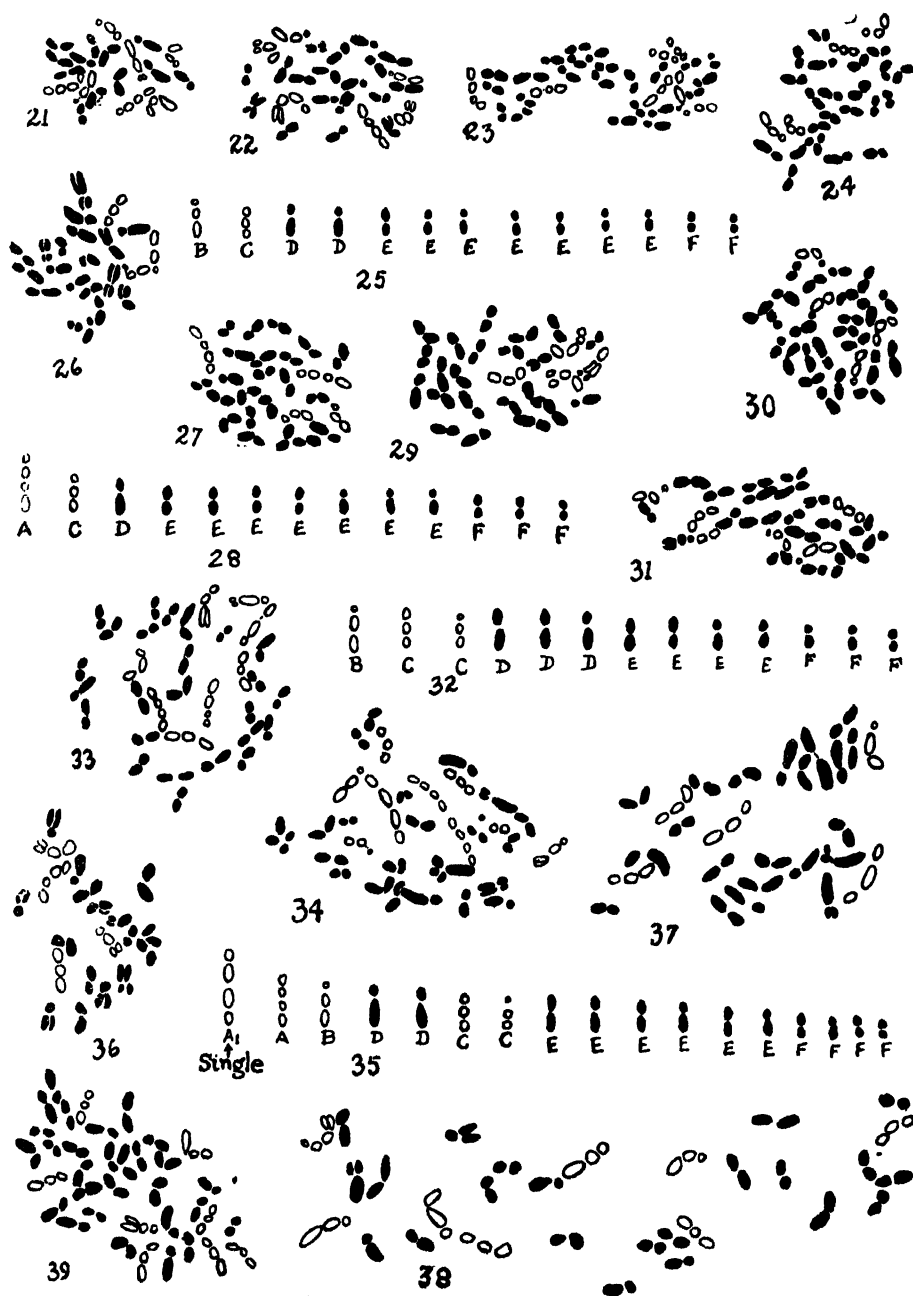
Fig. 4.—*C. leopardina*. Somatic prophase showing two chromosomes attached with the nucleolus.

Figs 5-7.—*C. princeps*. Normal somatic metaphase ( $2n = 11$ ), idiogram and variation plate with 20 chromosomes respectively.

Figs. 8-11a.—*C. vandenheckei*. Normal somatic metaphase ( $2n = 22$ ), idiogram, variation metaphase with 24 chromosomes, late separation and lagging chromosomes respectively.

Figs. 12-18a.—*C. zebrina*. Normal somatic metaphase ( $2n = 22$ ), idiogram, variation metaphase with 20, 23, 25, 26, 30 and 22 (structural alteration) chromosomes respectively.

Figs. 19-20.—*C. ornata*. Normal somatic metaphase ( $2n = 26$ ) and idiogram respectively.



TEXT-FIG. 2

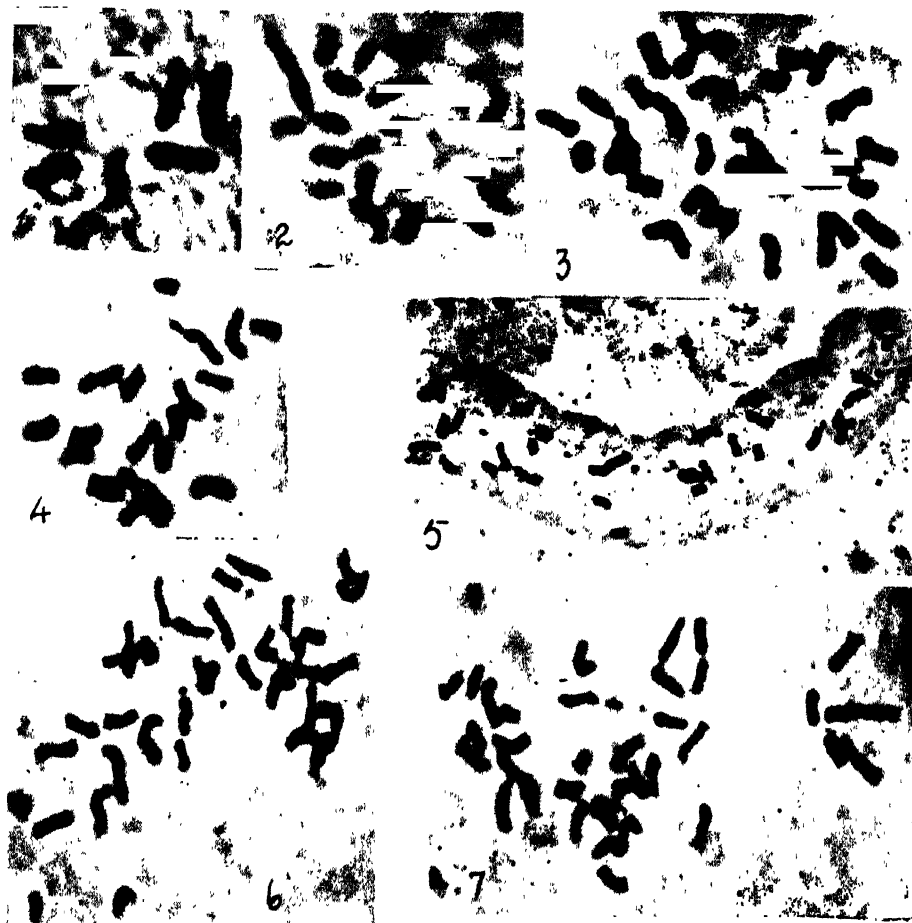
Figs. 21-23.—*Calathea ornata*. Variation metaphase with 19, 24 and 30 chromosomes respectively.

Figs. 24-26.—*Maranta leuconeura*. Normal somatic metaphase ( $2n = 26$ ), idiogram and variation metaphase with 22 chromosomes respectively.

Figs. 27-30.—*M. leuconeura*. Normal somatic metaphase ( $2n = 26$ ) idiogram, variation metaphase with 26 (structural alteration) and 28 chromosomes respectively.

Figs. 31-33.—*M. picta*. Normal somatic metaphase ( $2n = 26$ ), idiogram and variation metaphase with 33 chromosomes respectively.

Figs. 34-39.—*M. kegeljani*. Normal somatic metaphase ( $2n = 33$ ), idiogram, and variation plate with 20, 26, 30 and 36 chromosomes respectively.



Mp. 1. -*Galathea leopardina*. Normal somatic metaphase with 8 chromosomes.

Mp. 2. -*Maranta leuconera*. Normal somatic metaphase with 26 chromosomes.

Mps. 3-7. -*Maranta kegelani*. Somatic metaphase with 36, 20, 30, 36 and 33 chromosomes respectively.







TEXT-FIG. 3.

Figs. 40-45.—*Maranta kegeljani*. Variation metaphase with 41, 42, 44 and 47 chromosomes, lagging chromosomes and 33 chromosomes with structural alteration respectively.

# THE UTILIZATION AND SYNTHESIS OF OLIGOSACCHARIDES BY TWO SPECIES OF *PESTALOTIA*

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## ABSTRACT

The utilization of oligosaccharides by two species of *Pestalotia* viz., *P. banksiana* Cava (isolated from the diseased leaves of *Grevelia robusta*) and *P. citri* Mundk. and Koshwala (isolated from the diseased leaves of *Citrus grandis*) was studied. Chromatographic technique was used to detect the presence of various sugars formed during assimilation. Three sources of nitrogen viz., ammonium chloride, asparagin and potassium nitrate were separately supplied in combination with different oligosaccharides.

Raffinose, sucrose and maltose were used after hydrolysis. Only two sugars viz., melibiose and laevulose were obtained during the assimilation of raffinose. The laevulose fraction was utilized faster while the melibiose fraction persisted upto the 15th day.

Sucrose was a good source. Chromatographic results showed that its both the components viz., glucose and fructose were utilized by these fungi. *Pestalotia citri* assimilated glucose earlier than fructose. *P. banksiana* also behaved in the same manner when asparagin or  $\text{NH}_4\text{Cl}$  were used as nitrogen sources, but with potassium nitrate the assimilation of both glucose and fructose was almost simultaneous. *P. banksiana* synthesized an oligosaccharide (Rf 0.51) on sucrose-asparagin medium.

Maltose was the best sugar for both the organisms. These fungi converted maltose by transglucosidation into an oligosaccharide (malto-triose) with simultaneous liberation of glucose.

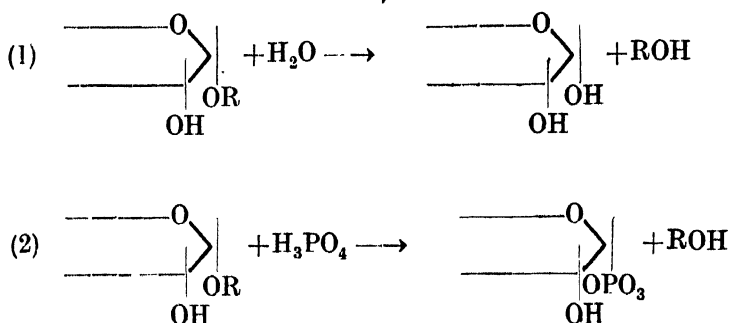
Cellobiose, lactose and melibiose were poorly utilized through a nonhydrolytic pathway. Melibiose (which is a component sugar of raffinose) influences the assimilation of raffinose.

Both the organisms preferred the ammonium nitrogen ( $\text{NH}_4\text{Cl}$ ), which was followed by asparagin and  $\text{KNO}_3$  respectively. It was observed that with certain exceptions the oligosaccharides or their hydrolytic products were assimilated slightly earlier if ammonium chloride was used as a source of nitrogen.

A combination of maltose- $\text{NH}_4\text{Cl}$  was best for both the organisms.

Oligosaccharides are complex sugars composed of two or more monosaccharide units linked together through a glycosidic linkage. Fungi generally utilize these complex sugars after the glycosidic linkage is broken and free monosaccharides are available in the medium. Two general mechanisms effect the cleavage of the glycosidic linkage in a biological system. The first involves the hydrolysis (1) and the second one involves the phosphorolysis (2) of the glycosidic bond. Both these reactions are catalysed by specific enzymes. Recent investigations of various investigators have shown that the pathways of assimilation of different oligosaccharides vary with the organism. Their results have also shown that the so-called "hydrolytic enzymes" which cause the hydrolysis of oligosaccharides (during the course of its assimilation by fungi) can also cause the replacement reaction of the glycosidic bond under suitable experimental conditions. They observed that the oligosaccharides were synthesized as intermediates during the enzymic hydrolysis of disaccharides by fungi. Our recent investigations with another

species of *Pestalotia* viz., *P. mangiferae* have also shown that the assimilation of sugars was also influenced by the type of the nitrogen source available in the



medium. It was, therefore, considered desirable to examine the effect of association of different nitrogen sources with various oligosaccharides.

#### MATERIALS AND METHODS

The diseased leaves of *Grevelia robusta* and *Citrus grandis* were repeatedly observed at various places at Allahabad. They were infected with *Pestalotia banksiana* and *P. citri* respectively. The two organisms were isolated from the respective hosts. Six oligosaccharides viz., (raffinose<sup>1</sup>, sucrose<sup>1</sup>, maltose<sup>1</sup>, lactose<sup>2</sup>, melibiose<sup>3</sup> and cellobiose<sup>1</sup>), and three nitrogen sources (viz., ammonium chloride<sup>1</sup>, asparagin<sup>2</sup> and potassium nitrate<sup>2</sup> were individually added to the basal medium (KH<sub>2</sub>PO<sub>4</sub> 1.75 gms, MgSO<sub>4</sub> 7H<sub>2</sub>O 0.75 gms, and double distilled water 1 litre) at a rate that supplied 4000 mgs. of carbon and 490 mgs. of nitrogen per litre respectively. Eighteen different combinations were prepared. Very thoroughly cleaned Pyrex glass ware were used. Equal quantities (25 ml.) of medium was dispensed into 150 ml. Erlenmeyer flasks. The media were fractionally sterilized for three consecutive days. Both the species of *Pestalotia* were daily inoculated at a fixed time for 15 days.

Three replicates were taken in each case. On the sixteenth day (when 1-15 day old cultures were available from different sets), the fungal mats from each set were separately filtered on Whatman No. 42 filter papers. The filtrate of each day was set aside for chromatographic analysis. The mycelial growth of sixth, eleventh and sixteenth days were dried and those dry weights served as quantitative measure of growth. The circular paper chromatographic technique (Ranjan *et al.*, 1955) was employed to detect the presence of various sugars formed during the assimilation of different oligosaccharides. n-butanol-pyridine-water (60:40:30) or n-butanol-acetic acid-water (4:1:5) were used as solvents and aniline-diphenylamine-phosphoric acid (Buchan and Savage, 1952) was used as spray reagent. The average R<sub>f</sub> values of various sugars (with BAH as solvent) are given at appropriate places in the text and in Table 3.

<sup>1</sup> B.D.H.

<sup>2</sup> E. Merck.

<sup>3</sup> DIFCO.

## EXPERIMENTAL

Table 1 showing the dry weight (in mgs.) of *Pestalotia banksiana* obtained on 6th, 11th and 16th days on different combinations of oligosaccharides and nitrogen sources.

TABLE 1

*Oligosaccharides*

| Nitrogen Source   | Days of incubation | Raffinose | Sucrose | Maltose | Cellobiose | Lactose | Melibiose |
|-------------------|--------------------|-----------|---------|---------|------------|---------|-----------|
| Amm. chloride     | 5                  | 30        | 48      | 60      | 10         | 20      | 8         |
|                   | 10                 | 52        | 86      | 106     | 22         | 42      | 15        |
|                   | 15                 | 64        | 108     | 144     | 28         | 54      | 20        |
| Asparagin         | 5                  | 22        | 60      | 56      | 8          | 20      | 7         |
|                   | 10                 | 40        | 106     | 98      | 18         | 38      | 12        |
|                   | 15                 | 52        | 132     | 126     | 24         | 50      | 14        |
| Potassium nitrate | 5                  | 16        | 40      | 48      | 8          | 12      | 6         |
|                   | 10                 | 28        | 68      | 84      | 14         | 21      | 10        |
|                   | 15                 | 38        | 86      | 102     | 19         | 30      | 12        |

Table 1 shows that *P. banksiana* attained best growth on a maltose— $\text{NH}_4\text{Cl}$  medium. The table also shows that with the exception of sucrose all other oligosaccharides were assimilated better when the ammonium nitrogen was available in the medium. Asparagin was, however, most suitable with sucrose though organic nitrogen (supplied by asparagin) was the next choice in all other combinations used in the present investigations. Nitrate nitrogen was not very suitable. The results also indicated that maltose and sucrose were best oligosaccharides. Raffinose and lactose could also support satisfactory growth when supplied with  $\text{NH}_4\text{Cl}$  or asparagin. Cellobiose and melibiose were definitely very poor oligosaccharides.

Table 2 showing the dry weight (in mgs.) of *P. citri* on 6th, 11th and 16th days on different combinations of various oligosaccharides and nitrogen sources.

TABLE 2

*Oligosaccharides*

| Nitrogen Source   | Days of incubation | Raffinose | Sucrose | Maltose | Cellobiose | Lactose | Melibiose |
|-------------------|--------------------|-----------|---------|---------|------------|---------|-----------|
| Amm. chloride     | 5                  | 11        | 30      | 38      | 4          | 20      | 4         |
|                   | 10                 | 26        | 52      | 66      | 7          | 32      | 8         |
|                   | 15                 | 34        | 69      | 84      | 9          | 41      | 10        |
| Asparagin         | 5                  | 12        | 26      | 8       | 6          | 10      | 6         |
|                   | 10                 | 23        | 44      | 56      | 11         | 17      | 12        |
|                   | 15                 | 30        | 57      | 78      | 15         | 22      | 17        |
| Potassium nitrate | 5                  | 4         | 24      | 30      | 2          | 8       | 3         |
|                   | 10                 | 17        | 42      | 48      | 4          | 14      | 5         |
|                   | 15                 | 24        | 50      | 64      | 7          | 17      | 8         |

It is obvious from Table 2 that in general the behaviour of *Pestalotia citri* was similar to that of *P. banksiana* but cellobiose and melibiose supported slightly



better growth when they were used in combination with asparagin than with ammonium chloride. The nitrate nitrogen was comparatively poorer than ammonium or organic nitrogen.

The average Rf values of various sugars and the results of the chromatographic investigations have been summarized\* in Table 3.

Table 3 showing the reaction of various oligosaccharides as well as the time of appearance of the hydrolytic products and their rate of utilization by *P. banksiana* and *P. citri*.

Table 3 shows that raffinose was utilized through a hydrolytic pathway by both the species of *Pestalotia*. Only two sugars were detected in the breakdown of this oligosaccharide viz., melibiose (Rf. 0.44) and fructose (Rf 0.70). Melibiose fraction could not be consumed completely by any of the two organisms, while the laevulose fraction was utilized. The rate of assimilation of laevulose greatly depended on the type of the nitrogen available in the substrate. Sucrose was also used after hydrolysis. Generally, both the organisms assimilated glucose earlier than fructose. In the presence of potassium nitrate *P. banksiana* utilized both the hydrolytic products (viz., glucose and fructose), simultaneously. This organism was also capable of synthesizing an oligosaccharide (Rf 0.49) with sucrose in presence of asparagin. Maltose was hydrolysed to glucose before assimilation. A simultaneous synthesis of maltotriose Rf. 0.22 (an oligosaccharide) was also observed during the utilization of this sugar. In every case this synthetic product appeared as an intermediate and was not dependent on the source of nitrogen. The above table also shows that the remaining oligosaccharides (viz., lactose, melibiose and cellobiose) were utilized through a non-hydrolytic pathway. Except lactose in combination with ammonium chloride, *P. citri* could not finish any of these three oligosaccharides even in 15 days. *P. banksiana* was slightly faster in consuming these oligosaccharides, though cellobiose and melibiose were not used up completely when supplied in combination with potassium nitrate.

## DISCUSSION

Neuberg and Mandl (1950) reported that invertase\* hydrolyses raffinose into fructose and melibiose only, though normally it can be hydrolysed into 5 products (viz., sucrose, melibiose, glucose, fructose and galactose). In the present investigations only laevulose and melibiose have been noticed and thus it appears that invertase is secreted by both the species of *Pestalotia*. This enzymic system also appears to function during the assimilation of sucrose, as both glucose and fructose were found to be present. *P. banksiana* exhibited slightly different behaviour on a sucrose-asparagin medium because besides the hydrolytic products of sucrose an oligosaccharide (Rf 0.49) was also synthesized in the medium on the 3rd and 4th days. Synthesis of a similar oligosaccharide from sucrose by *Aspergillus flavus* and *A. niger* has been reported by Giri *et al.* (1954) and Bacon and Bell (1953) respectively. Bealing and Bacon (1953) using enzymic extract of *Aspergillus niger* attributed the breakdown of sucrose to the transference of fructose residues to suitable acceptors by a  $\beta$ -fructofuranosidase. This interpretation explains the synthesis of an intermediate oligosaccharide during the hydrolysis of sucrose. Giri *et al.* (1954) who were able to separate this oligosaccharide have established that the oligosaccharide in question was a trisaccharide. They further found that on acid hydrolysis this oligosaccharide was hydrolysed to fructose and glucose which existed in the ratio of 2:1.

Oligosaccharide formation by transglycosidation with enzymes from *Aspergillus oryzae* has also been reported by Pazur (1954). Edelman and Bealing (1953) have

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\* It has also been termed as succharase, sucrase and  $\beta$ -fructosidase.

suggested that glycosidases (usually considered to be merely catalysts of glycosidic bonds), can also catalyse replacement reaction. There is considerable controversy in literature about the identity or non-identity of various enzymic materials. Recent researches of various biochemists suggest that the cleavage of maltose was not accomplished by a hydrolytic mechanism but by a transglycosidation leading to the formation of oligosaccharides. However, the discovery of these enzymes is extremely important as they demonstrate that the synthesis of the glycosidic bond in all living systems does not involve the direct participation of the phosphate.

The authors in their previous investigation (1958) had observed that the utilization of two identical oligosaccharides may be accomplished through two different pathways by a particular organism. The present results also confirmed the previous observations which showed that maltose and cellobiose (which are identical in structure except for the mode of linkage between two glucose units) were not only used through two different pathways, but their availability was totally different for both the species of *Pestalotia*. The former was found to be the best source of oligosaccharide while the latter was very poorly and slowly utilized. On the basis of present results it can also be interpreted that probably the fungi under study were incapable of synthesizing  $\beta$ -glucosidase and so they were unable to hydrolyse cellobiose (which has a  $\beta$ -glucosidic link).

The results also reveal that *P. citri* could attain only negligible growth on raffinose- $\text{KNO}_3$  or maltose- $\text{NH}_4\text{Cl}$  media till the 6th day. A glance at Table 3 shows that the hydrolytic products of raffinose or maltose were not detected chromatographically till that day. This further showed that brisk mycelial growth started only after the production of the hydrolytic products.

None of the two organisms were able to utilize melibiose satisfactorily. Chromatographic results indicated that even the melibiose fraction formed during the assimilation of raffinose was not assimilated and it remained accumulated till the 15th day. The comparatively poor results with raffinose appear to be connected with the unsatisfactory utilization of this substance (melibiose). The present investigations also revealed that though lactose was assimilated by both the organisms, through a nonhydrolytic pathway, yet it was comparatively a better source than either cellobiose or melibiose, which were also used up through a nonhydrolytic pathway.

The present species of *Pestalotia* behaved like *P. mangiferae* (Bilgrami, 1956) as it was found that ammonium nitrogen was more suitable than nitrate nitrogen.

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# LOCATION OF THE OLFACTORY RECEPTORS OF THE BLOWFLY *PHORMIA REGINA* MEIGEN

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## ABSTRACT

The site of olfactory receptors of the blowfly *Phormia regina* Meigen and the olfactory rôle of its antennal receptors have been experimentally determined.

The capacity of nine essential oils to stimulate olfactory receptors of the blowfly has been tested and the oil of Caraway, being the best stimulant, has been employed for the present work. The response of the blowfly to vapours of this oil is not due to chemical irritation.

Olfactory receptors in *Phormia regina* are located on the antennae, labellum, and, tarsi but not on the palpi. Comparative sensitivity of the different appendages to the oil vapours has been shown. A threshold population of the olfactory receptors is necessary for the maximum degree of olfactory response.

As regards the rôle of antennal receptors, the present experiments demonstrate that, in a stationary blowfly, surface-cones are responsible for the perception of the oil-vapours.

## INTRODUCTION

The location and structure of olfactory receptors of blowflies have been studied by several workers but their conclusions are not in agreement with one-another. McIndoo (1933, 1934) studied the structure and distribution of these organs in *Calliphora erythrocephala*, *Lucilia sericata* and *Phormia regina* and he concluded that the olfactory receptors are situated on the bases of the wings and legs but none on the head. On the other hand, Hartung (1935) contended that the olfactory organs of *Calliphora erythrocephala* are restricted to the antennae only. Abbot's (1938) experiments with *Cynomyia cadavarina* and *Lucilia sericata* indicated that the olfactory organs are definitely located on the head and also on other parts of the body. According to Frings (1941), however, the antennae and the labellum are the only loci of olfactory receptors in the blowfly *Cynomyia cadavarina*. In *Phormia regina* these receptors are reported to occur on the antennae, labellum and palpi (Dethier, 1952).

There is also some uncertainty regarding the identity and structure of the olfactory receptors of blowflies. Detailed structure of the olfactory pores was described by McIndoo (1934) who believed these pores as olfactory organs. But his view was disproved by later experimental work. The sensory structures of the antennae and palpi of certain species of blowflies have also been studied by various workers, particularly by Smith (1919), McIndoo (1934) and Liebermann (1926). Two types of sensory receptors have been found to occur on the antennae of blowflies : (i) *Sensilla basiconica* or surface-cones (*flächenkegel* of Liebermann, 1926) and, (ii) *Sensilla coeloconica* or pit-cones (*grübenkegel* of Liebermann, 1926). Of these, the receptors in the pits have been generally regarded as the olfactory end-organs, but no experimental evidence in support of this view has so far been adduced.

The present investigations were taken up in order to determine the site of olfactory receptors of the blowfly *Phormia regina* Meigen and, also, to demonstrate the rôle of antennal sensory structures. This work was done in the Department of Biology, John Hopkins University, Baltimore (Md.).

## MATERIALS AND METHODS

Freshly emerged adults of *Phormia regina* Meigen were used for the present work. The technique for measuring the olfactory response of the blowflies was essentially the same as that adopted by Abbot (1932, 1938) and Frings (1941). The blowflies were singly mounted along their backs on waxed sticks. Each blowfly was brought near the source of the odour and its proboscis-response was noted. If the odour was acceptable and attractive, the fly would respond by extending its proboscis in an attempt to feed on the stimulant (Abbot, 1932; Frings, 1941).

The first step in the present work was to select a suitable olfactory stimulant which would cause the blowflies to extend the proboscis. Fermented casein, malt extract, putrid meat and putrid eggs have been found to attract free moving blowflies in an olfactometer (McIndoo, 1934); but these substances failed to evoke proboscis response of the mounted blowflies used in the present work. Abbot (1938) tested the stimulating effect of various essential oils on the proboscis-response of mounted blowflies, *Lucilia sericata* and *Cynomyia cadavarina*. He stated that tansy oil was the best attractant. Frings (1941) trained the mounted blowflies (*Cynomyia cadavarina*) to extend the proboscis in response to stimulation by the vapours of coumarin. Dethier (1952) recorded the repellent effect of pentanol on *Phormia regina* in an olfactometer. In the present work it was considered desirable to take the proboscis-response of stationary blowflies as the criterion of olfactory perception and for this purpose it was necessary to select a suitable stimulant.

Since there is no record of the capacity of essential oils to stimulate chemoreceptors of *Phormia regina*, the following oils were tested: Oils of Caraway, Carvacrol, Celery seed, Parsley, Fennel, Rue, Dill seed, Phellandrene, and Coriander. These were obtained from Dodge and Olcott Company, New York. Each of the oils was placed in a glass vial and mounted blowflies were held, one by one, over the open mouth of the glass vial at a distance of two inches from the oil for thirty seconds and the proboscis-response was noted. For each oil fifty individuals were tested and the observations are recorded in Table I. Oil of Caraway was found to be the best stimulant. It may be noted that vapours of ethyl acetate, ethanol

TABLE I

*Proboscis-response of Phormia regina to essential oils*

| Name of oil | Percentage<br>of flies<br>responding | Name of oil  | Percentage<br>of flies<br>responding |
|-------------|--------------------------------------|--------------|--------------------------------------|
| Caraway     | 96                                   | Fennel       | 80                                   |
| Carvacrol   | 75                                   | Rue          | 40                                   |
| Celery seed | 40                                   | Dill seed    | 80                                   |
| Parsley     | 36                                   | Phellandrene | 50                                   |
| Coriander   | 16                                   |              |                                      |

and a few other organic solvents also stimulated the blowflies to extend their proboscis. But this response was not sustained and evidently due to chemical irritation since it was obtained even after the removal of all the appendages like the antennae, palpi, labellum and tarsi. On the other hand, oil of Caraway failed to evoke proboscis-response from the blowflies which were deprived of all these appendages. Also, on allowing the extended proboscis to come in contact with the oil the latter was ingested, though the blowfly died soon after. These observa-

tions indicate that oil of Caraway served as an olfactory stimulant and not as a chemical irritant. This oil was therefore employed in all the subsequent experiments.

In order to determine the site of olfactory receptors care was taken to maintain the blowflies in the same physiological state throughout the course of work. Freshly emerged blowflies were first given an opportunity to feed on 0.1 M sucrose solution for about 12 hours and, then, they were mounted on waxed sticks under CO<sub>2</sub> anaesthesia. After the blowflies recovered they were allowed to drink water to satiety so that they showed no response to water vapour. The proboscis-response of each of the blowflies to oil of Caraway was tested. *Only the individuals showing positive response were selected for further experiments* and they will be referred to as 'normal' blowflies. The flies which did not give any response were discarded.

The normal blowflies were divided into nine groups of ten each. Individuals in different groups were deprived of their appendages singly or in different combinations, as indicated in Table II, and their proboscis-response to the oil-vapour was again tested. Prior to removal of appendages the blowflies were fed on sucrose solution for two hours and, after the operation, they were allowed 12–20 hours to recover from the shock. They were allowed to drink water to satiety just before their response to the oil was determined. A period of 12 hours was found to be quite sufficient for recovery of the flies from the shock of operations. This series of experiments was repeated with five different batches of blowflies, all the individuals of a batch being the progeny of a single pair of parents drawn from a standard culture. The observations are recorded in Table II.

In order to ascertain the olfactory function of the antennal receptors the blowflies were deprived of all the appendages except the antennae and their response to the oil vapour was tested. Only the blowflies showing positive response were selected for the present experiment while others were discarded. These blowflies were divided into four groups. In one group both the antennae of each blowfly were completely coated with a layer of India Ink (waterproof) by means of a very fine brush. The blowflies of the second group were deprived of both their antennae. In the third group the pit-bearing areas on the undersurfaces of the antennae were similarly coated with India Ink. In the fourth group the surface-cones were sealed by smearing the outer surfaces and the terminal parts of the undersurfaces of the antennae with the ink. A small number of the surface-cones, situated in between the pits, was, however, left exposed. After allowing the blowflies to recover from the shock they were fed on water to satiety and then tested for their response to the oil vapours as before. The olfactory perception was measured in terms of percentage of the blowflies giving positive response.

#### DISTRIBUTION OF OLFACTORY RECEPTORS ON THE BODY

The olfactory responses of the blowflies deprived of their appendages in different combinations are indicated in Table II. Since only those normal blowflies which gave initial positive response were employed for the present work, the effect of the removal of any one or more appendages on the olfactory response of the flies would be quite definite.

The table shows that the olfactory response of the blowflies is completely abolished when all the four sets of appendages—antennae, palpi, labellum and tarsi—are removed (group IX). This suggests that centres for perception of the oil vapours are present on one or more of the amputated appendages.

In the blowflies which are deprived of the palpi but which retain antennae, labellum and tarsi (group I), the olfactory response is not in the least affected. On the other hand when all the appendages excepting the palpi are removed (group VII) the response of the blowflies is almost completely abolished. These

observations clearly indicate that no receptors on the palpi are involved in the perception of the vapours of oil of Caraway.

TABLE II

*Olfactory-response of blowflies deprived of their appendages in different combinations*

| Group No. | Receptor areas remaining      | Receptor areas removed                 | Experiment No. | No. of blowflies tested | No. of blowflies responding | Average percentage of blowflies responding |
|-----------|-------------------------------|--|----------------|-------------------------|-----------------------------|--|
| I         | antennae<br>labellum<br>tarsi | palpi                                  | i              | 10                      | 10                          | 100  |
|           |                               |  | ii             | 10                      | 10                          |  |
|           |                               |  | iii            | 10                      | 10                          |  |
|           |                               |  | iv             | 10                      | 10                          |  |
|           |                               |  | v              | 10                      | 10                          |  |
| II        | palpi<br>labellum<br>tarsi    | antennae                               | i              | 10                      | 9                           | 84   |
|           |                               |  | ii             | 10                      | 9                           |  |
|           |                               |  | iii            | 10                      | 8                           |  |
|           |                               |  | iv             | 10                      | 8                           |  |
|           |                               |  | v              | 10                      | 8                           |  |
| III       | antennae<br>palpi<br>tarsi    | labellum                               | i              | 10                      | 7                           | 70   |
|           |                               |  | ii             | 10                      | 6                           |  |
|           |                               |  | iii            | 10                      | 7                           |  |
|           |                               |  | iv             | 10                      | 7                           |  |
|           |                               |  | v              | 10                      | 8                           |  |
| IV        | antennae<br>palpi<br>labellum | tarsi                                  | i              | 10                      | 10                          | 96   |
|           |                               |  | ii             | 10                      | 10                          |  |
|           |                               |  | iii            | 10                      | 9                           |  |
|           |                               |  | iv             | 10                      | 9                           |  |
|           |                               |  | v              | 10                      | 10                          |  |
| V         | antennae                      | palpi<br>labellum<br>tarsi             | i              | 10                      | 7                           | 66   |
|           |                               |  | ii             | 10                      | 7                           |  |
|           |                               |  | iii            | 10                      | 6                           |  |
|           |                               |  | iv             | 10                      | 6                           |  |
|           |                               |  | v              | 10                      | 7                           |  |
| VI        | labellum                      | antennae<br>palpi<br>tarsi             | i              | 10                      | 7                           | 70   |
|           |                               |  | ii             | 10                      | 8                           |  |
|           |                               |  | iii            | 10                      | 7                           |  |
|           |                               |  | iv             | 10                      | 6                           |  |
|           |                               |  | v              | 10                      | 7                           |  |
| VII       | palpi                         | antennae<br>labellum<br>tarsi          | i              | 10                      | 0                           | 4  |
|           |                               |  | ii             | 10                      | 0                           |  |
|           |                               |  | iii            | 10                      | 0                           |  |
|           |                               |  | iv             | 10                      | 2                           |  |
|           |                               |  | v              | 10                      | 0                           |  |
| VIII      | tarsi                         | antennae<br>labellum<br>palpi          | i              | 10                      | 5                           | 48   |
|           |                               |  | ii             | 10                      | 3                           |  |
|           |                               |  | iii            | 10                      | 4                           |  |
|           |                               |  | iv             | 10                      | 6                           |  |
|           |                               |  | v              | 10                      | 6                           |  |
| IX        | nil                           | antennae<br>labellum<br>palpi<br>tarsi | i              | 10                      | 0                           | 0  |
|           |                               |  | ii             | 10                      | 0                           |  |
|           |                               |  | iii            | 10                      | 0                           |  |
|           |                               |  | iv             | 10                      | 0                           |  |
|           |                               |  | v              | 10                      | 0                           |  |

On removal of both the antennae (group II) or all the tarsi (group IV) there is a slight fall in the olfactory response of the blowflies. However, when only the antennae (group V) or the tarsi (group VIII) are retained and the remaining appendages removed, there is a sharp fall in the olfactory response of the blowflies; but, still the perception of the oil vapours is quite marked (66 per cent for the blowflies with antennae and 48 per cent for those with tarsi only). This shows that receptors for the perception of oil of Caraway are located on the antennae and tarsi; but the antennae are more sensitive than the tarsi.

In the case of the blowflies which are deprived of the labellum alone (group III) there is a marked fall in the olfactory response from 100 per cent to 70 per cent. When labellum alone is retained and all the other appendages are removed (group VI) the response is almost of the same order as that with the labellum removed but antennae and tarsi retained. These observations indicate that labellum also bears the olfactory receptors.

### OLFACTORY RECEPTORS OF THE ANTENNAE

The sensory structures of the antennae of the blowflies *Lucilia caesar*, *Calliphora erythrocephala*, *Calliphora vomitoria*, *Cynomyia mortuora* etc., have been studied in detail by Liebermann (1926), Smith (1919) and McIndoo (1934). Two types of receptors have been recognised: (i) *Sensilla cocloconica* (Berlese, 1909) or pit-cones (*Grübenkegel* of Liebermann, 1926) which are the sensory hairs present within simple or complex pits on the antennae; and, (ii) *Sensilla basiconica* (Berlese,

TABLE III

*Olfactory response of blowflies with different parts of the antennae sealed with India Ink*

| Group No. | Receptor areas remaining | Receptor areas sealed  | Experiment No. | No. of blowflies tested | No. of blowflies responding | Average percentage of blowflies responding |
|-----------|--------------------------|------------------------|----------------|-------------------------|-----------------------------|--|
| I         | nil                      | entire antennae sealed | i              | 10                      | 0                           | 4  |
|           |                          |                        | ii             | 10                      | 0                           |  |
|           |                          |                        | iii            | 10                      | 1                           |  |
|           |                          |                        | iv             | 10                      | 1                           |  |
|           |                          |                        | v              | 10                      | 0                           |  |
| II        | nil                      | antennae removed       | i              | 10                      | 0                           | 2  |
|           |                          |                        | ii             | 10                      | 1                           |  |
|           |                          |                        | iii            | 10                      | 0                           |  |
|           |                          |                        | iv             | 10                      | 0                           |  |
|           |                          |                        | v              | 10                      | 0                           |  |
| III       | surface-cones            | pit-cones              | i              | 10                      | 8                           | 78   |
|           |                          |                        | ii             | 10                      | 7                           |  |
|           |                          |                        | iii            | 10                      | 10                          |  |
|           |                          |                        | iv             | 10                      | 9                           |  |
|           |                          |                        | v              | 10                      | 5                           |  |
| IV        | pit-cones                | surface-cones          | i              | 10                      | 1                           | 20   |
|           |                          |                        | ii             | 10                      | 0                           |  |
|           |                          |                        | iii            | 10                      | 5                           |  |
|           |                          |                        | iv             | 10                      | 4                           |  |
|           |                          |                        | v              | 10                      | 0                           |  |

*Note* : Only those blowflies were used in this series of experiments which, on removal of their labella, palpi and tarsi, gave positive proboscis-response to the vapours of oil of Caraway.

1909) or surface-cones (*Flächenkegel* of Liebermann) which are the sensory hairs present on the surfaces of the antennae. The number, arrangement and size of the surface-cones and the pit-cones differ in different species of the Muscid flies. In *Phormia regina*, also, the same two types of sensory structures are found on the antennae. The pits are present on the proximal two-thirds part of the under-surface of each antenna along its lateral edges. The surface cones are present over the entire outer surface and on the terminal part of the undersurface of the antenna; some are also present on the area inbetween the pits.

In order to determine the rôle of these structures in the perception of odour, one or the other or both types of the receptors were sealed and the olfactory response of the treated blowflies was measured as described before. The observations are given in Table III. It is clear that the response of the blowflies with completely sealed antennae (group I) and of those with the antennae removed (group II) is almost completely abolished. This shows that the antennal receptors can be effectively sealed with India Ink so that they cannot be stimulated by the oil vapours.

When the pit-bearing areas of the antennae are sealed (group III) and greater proportion of the surface-cones are exposed, olfactory response of the blowflies, though suffering a slight fall, is still very high (78 per cent); the slight fall in the response may be due to the sealing of some of the surface-cones present in the pit-bearing areas. This experiment shows that the surface-cones are definitely the centres for registering odorous stimuli. On the other hand, when the pits are kept exposed and the surface-cones are mostly sealed (group IV) olfactory response of the blowflies falls sharply from 100 per cent to 20 per cent, which suggests that the antennal pits are not able to perceive vapours of the essential oil. Slight response which is obtained with flies of this group may be due to the few exposed surface-cones present inbetween the pits.

#### DISCUSSION

The observations recorded in the present paper demonstrate that receptors for the perception of vapours of the oil of Caraway are located on the antennae, labellum and tarsi of the blowfly *Phormia regina*, but not on the palpi.

Whether the response of the blowflies to the oil vapours is due to the stimulation of their olfactory receptors or due to irritation of the common chemical sense, needs consideration. According to Valentine (1931) and Marshall (1935) strong vapours of certain chemicals, particularly essential oils, do not stimulate olfactory receptors but cause irritation to insects through their action on the common chemical sensory cells present all over the body. Valentine demonstrated that the beetles (*Tenebrio molitor*), in which all possible centres of olfactory or gustatory receptors had been removed or sealed, still reacted to the essential oils, suggesting that such a response cannot be due to olfaction but to the stimulation of common chemical sense. Two important facts which emerge from the present work on *Phormia regina* rule out the possibility that vapours of oil of Caraway irritate the blowflies through their action on the common chemical sense: (i) Response of the blowflies is completely abolished when antennae, labellum and tarsi are removed, but remaining portions of the legs and the palpi, which are known to bear sensory hairs, are retained. If the common chemical sense was involved the amputated blowflies should have continued to respond to the oil vapours. (ii) The fact that the blowflies, when their extended proboscis is allowed to come in contact with the oil, actually ingest the stimulant, goes against the view that the flies are being irritated. They do not make any attempt to ingest ethyl acetate or ethanol, vapours of which evoke proboscis-response from the blowflies and this response persists even after removal of the appendages. In strong concentrations the oil of Caraway may serve as a repellent but not as a chemical irritant.

It is therefore certain that some specialised receptors are concerned with the perception of the oil of Caraway. Since these receptors are stimulated from a distance, it is quite reasonable to regard them as olfactory receptors.

The observation that the palpi of *Phormia regina* are not sensitive to the oil vapours is in conflict with Dethier's (1952) observations that the palpi of this species are able to perceive the repellent vapours of pentanol. Chief difference in the present work and that of Dethier lies in the nature of the stimulant employed. The discrepancy in Dethier's results and those of the present study seems to suggest that different types of chemical stimuli may stimulate different sets of chemoreceptors and receptors on the palpi may be sensitive to pentanol and other similar repellents but not to the essential oils. This point needs further investigation.

The fact that the antennae and labellum, and not the palpi, bear olfactory receptors in *Phormia regina* agrees with Frings' conclusions on *Cynomyia cadaverina*. But there is a difference in that Frings did not find the tarsi to be sensitive to coumarin vapours. This difference may again be due to differences in the chemical stimuli or in the species. However, it may be remarked that gustatory receptors have also been regarded to perceive strong vapours of certain chemicals from a distance (McIndoo, 1934; Marshall, 1935). It is not yet certain whether the sensory structures of the tarsi of *Phormia* responsible for the perception of the oil vapours are identical with or distinct from the gustatory chemoreceptors.

Another point which merits attention is the comparative sensitivity of different appendages to the vapours of oil of Caraway. It has been observed that the olfactory response of the blowflies is only slightly affected when their tarsi are removed. This might imply that these appendages do not contribute towards registering the olfactory stimuli. But when only the tarsi are retained appreciable response (48 per cent) is again obtained, showing the presence of the receptors on these organs. Similarly when antennae alone are removed there is a fall in the response of the blowflies from 100 per cent to 84 per cent while the removal of labellum alone results in a fall to 70 per cent. This apparently suggests that labellum is more sensitive than the antennae. But olfactory response of the blowflies which retain only the antennae (66 per cent) is almost of the same order as that of the blowflies retaining the labellum alone (70 per cent). These results can be explained on the basis of a presumption that a certain minimum (threshold) number of olfactory receptors is required for the maximum degree of perception. The degree of olfactory response is directly proportional to the number of receptors stimulated. When either antennae or tarsi are removed the number of receptors on the remaining appendages may be just sufficient to compensate for the loss of some receptors and that is why the response is not appreciably reduced. On the other hand, when labellum alone is removed, a sharp fall in response suggests that the receptor population is markedly decreased and receptors on the antennae and tarsi fall too short of making up the deficiency. Similarly, when both the antennae and tarsi are removed and labellum alone is retained, fall in response is again sharp since the receptor population on labellum is much too short of the threshold. Fall in response of blowflies retaining only the antennae or only the tarsi can also be explained on the same basis.

As regards the rôle of antennal receptors in olfaction, pit-cones or the *sensilla coeloconica* are believed to be the olfactory end-organs by most of the workers (Röhler, 1906; Smith, 1919; Liebermann, 1926) but there has been no experimental evidence to support this view. On the basis of comparison between the biology of the Muscid flies and the condition of their antennal receptors, Liebermann (1926) suggested that the surface-cones (*sensilla basiconica* or *flächenkegel*) are capable of perceiving odour from nearby stimuli when the fly is resting and its antennae are in repose. In this condition the pit-cones do not appear to perceive the odour unless strong wind is blowing and the stimulant is directly below the antennae. He suggested that the pit-cones perceive odour when the fly is flying



and its antennae are held erect. The present experiments demonstrate that the surface-cones definitely perceive odour. The lack of perception by the pit-cones of the mounted blowflies, even when air is blown across the open end of the oil-containing vial by a fan, does not conclusively show that these receptors are not olfactory in function. For, if Liebermann's view is correct, the pits of stationary flies would not register any olfactory stimulus. It is desirable to test the olfactory function of the pit-cones in flying blowflies.

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## ADDITIONS AND CORRECTIONS TO THE INDO-NEPALESE FLORA

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### ABSTRACT

This paper records one new species and one new variety from Saurashtra, two new varieties from East Nepal contributed by the author jointly with M. L. Banerji, and several notes on Bombay Orchids contributed by the author with Z. Kapadia.

Of the new taxa of plants listed below, two have been collected personally by the author in Saurashtra ; the others have come to light in the course of studies done by research students under his guidance. The plants of East Nepal have been collected by Shri M. L. Banerji during his long term of botanical exploration in that country ; the orchids have been collected by the author and Shri Z. Kapadia, who has been working on the Orchids of Bombay for several years. Both M. L. Banerji and Z. Kapadia have been helped in their work through the kindness of the administrators of the Sir Dorabji Tata Trust, and wish me to put this on record in these pages as a token of their gratitude.

***Tephrosia jamnagarensis*** Santapau, spec. nov. (Text-fig. 1).

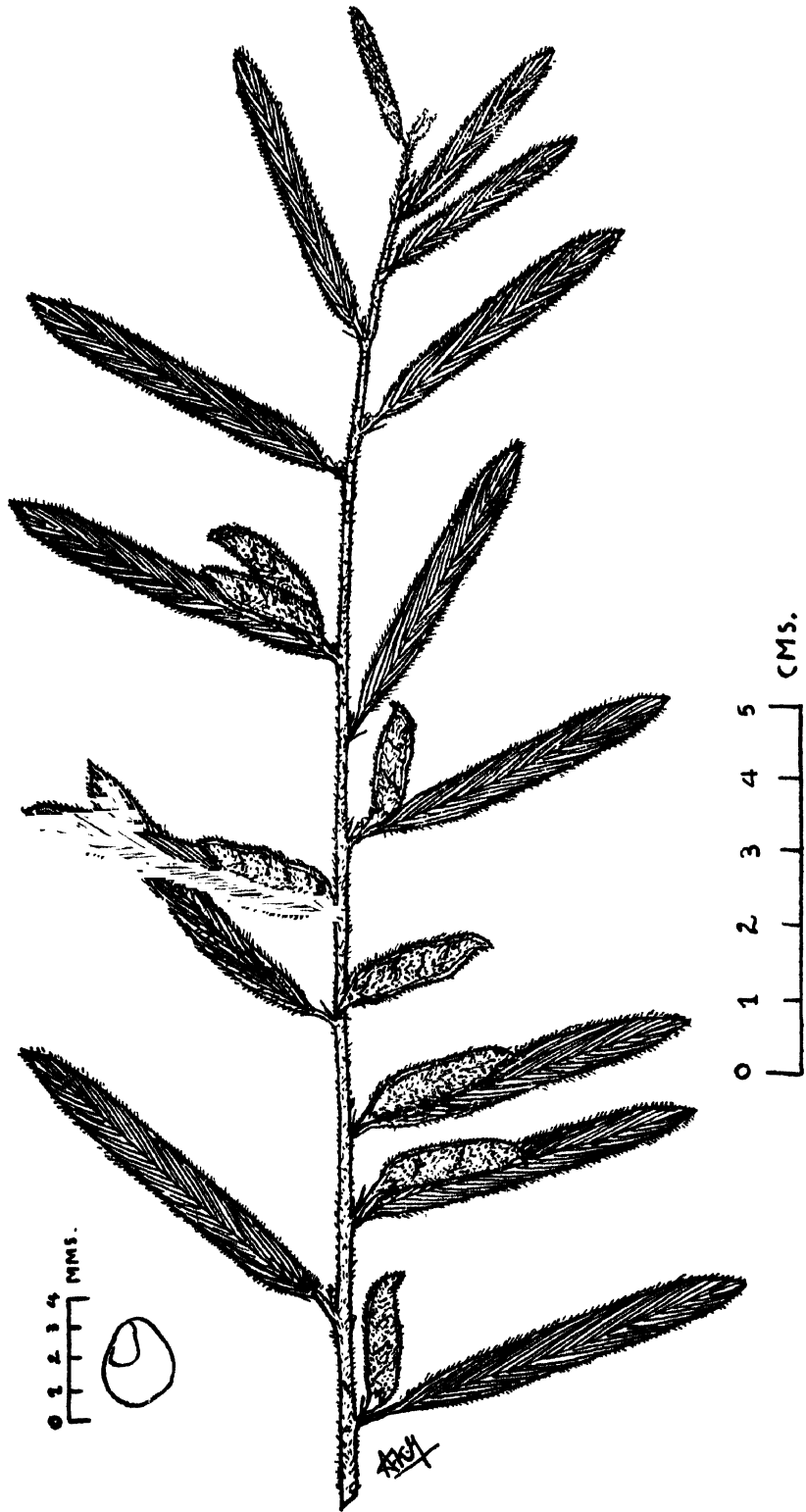
Accedit ad *Tephrosiam strigosam* Sant. & Mahesh. (*T. tenuem* Wall.) habitu generali, a qua tamen differt caractere foliorum, magnitudine et forma leguminum et brevitate pedunculorum.

Herba vel suffrutex erectus vel suberectus, annuus (?), vix ramosus. *Caulis* pilis albidis, adpressis ornatus. *Folia* simplicia, 3-5.8 cm. longa, 4-7 mm lata, linearia, in pagina superiore glabra vel subglabra, pilis nonnullis sericeis, patentibus longisque ornata, in inferiore pagina dense pilosa pilis argenteis adpressisque, apice subobtusum, distincte apiculatum, basi acuta ; nervi laterales ca. 25-30, inter se paralleli, margine integro, duplici nervo a basi ad apicem decurrente prope marginem, distincti in pagina superiore, pilis operiti sed distincti etiam in inferiore. *Petiole* 2-3 mm. longi, valde pilosi ; stipulae subulatae, 3-4 mm. longae, pilosae. *Flores* singuli vel bini ad omnes fere axillas foliorum ; pedunculi aequae longi ac petioli, vel hisce breviores, dense pilosi. *Calyx* dense pilosus, 2-3 mm. longus, plus minusve ad mediam partem divisus, dentibus subulatis, filiformibus, pilosis, subaequalibus. *Corolla* non visa. *Legumen* complanatum, ca. 20×5 mm., dense pilosum pilis griseis, patentibus, obliquum ad utrumque apicem, apiculatum ; semina 5-6, reniformia, haud nitentia, brunnea.

Typus lectus inter gramina fructificans ad locum Rozi prope urbem Jamnagar, in provincia Saurashtra, die 16 octobris anni 1945 et positus in *Blatter Herbario*, in urbe Bombay sub numero *Santapan 7522*.

In many respects this plant is similar to *T. strigosa* Sant. & Mahesh. which in our floras goes under the name of *T. tenuis* Wall., from which it differs mainly in the type of leaves, the size and form of the legumes and the brevity of the peduncles.

Erect to suberect herb or undershrub, annual (?), sparsely branched. *Stems* simple or nearly so, covered with whitish adpressed hairs. *Leaves* simple, 3-5.8×0.4-0.7 cm., linear, glabrous above or subglabrous with a few silky spreading hairs, densely hairy with silvery adpressed hairs beneath, sub-obtuse and clearly apiculate at the apex, the base acute ; lateral nerves 25-30 pairs,



TEXT-FIG. 1. *Tephrosia jamnagarensis* Santapau.

parallel among themselves ; margins entire, with a nerve running from near the base to the apex very close to the margins ; the nerves are clear on the upper surface, covered with hairs but nearly equally distinct on the lower surface. *Petioles* 2-3 mm. long, very hairy ; stipules subulate 3-4 mm. long, very hairy. *Flowers* single or in pairs at practically all the axils of the leaves ; peduncles about as long as or slightly shorter than the petioles, densely hairy. *Calyx* very hairy, 2-3 mm. long, the teeth subulate, filiform, hairy, subequal. *Corolla* not seen. *Legume* or pod compressed, about  $20 \times 5$  mm., densely hairy with greyish, patent hairs, oblique at both ends, apiculate ; seeds 5-6, reniform, dull or mat, brownish.

The type of this new species was collected at Rozi near Jamnagar in Saurashtra among grasses, on the 16 October 1945 and has been deposited in the Blatter Herbarium, Bombay, under the number *Santapau* 7522.

This is a very distinct species ; the underside of the leaves is clearly silvery or densely argenteo-canescens, much more so than any of the specimens of *T. strigosa* from Saurashtra ; the peduncles are fairly stout, not filiform as in the other species, and generally shorter than the petioles. The legumes are about twice as broad and much more hairy than in *T. strigosa*, the seeds not separated by any internal partition. Rozi is the port of the city of Jamnagar ; the plant has been named *jamnagarensis* in token of gratitude for the constant help and encouragement of H. H. the Jam Saheb of Nawanagar, under whose auspices the botanical exploration of Saurashtra was started in 1945.

***Indigofera articulata* Gouan var. *monosperma* Santapau, var. nov. (Text-fig. 2).**

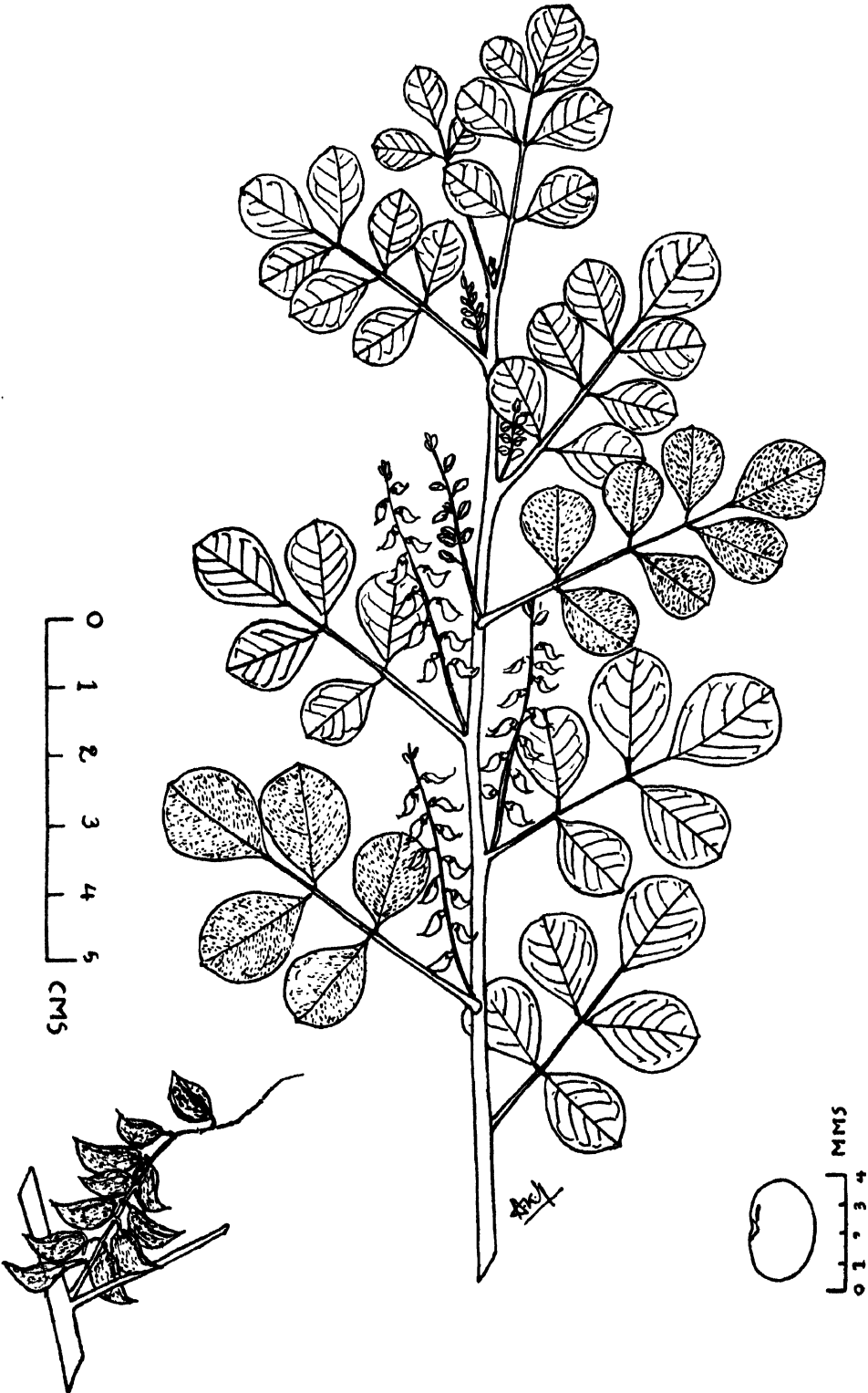
Accedit ad varietatem typicam, a qua tamen differt primo intuitu leguminibus monospermis, raro dispermis, numquam vero polyspermis.

*Suffrutex* erectus, plus minusve albidus pilis minutissimis adpressis, caulibus atque ramis subteretibus vel angularibus. *Folia* 7-10 cm. longa, stipulis minutis persistentibus ; foliola 7-9 numero in singulis foliis, opposita, obovata vel sub-orbicularia, rotundata vel paulo emarginata ad apicem, acuta vel cuneata ad basin, petiolulis 2 mm. longis, glabra supra, plus minusve dense hispidula pilis albidis adpressis infra, omnia subaequalia, vel foliolum terminale paulo largius caeteris. *Flores* axillares, racemosi, usque 50 in singulis racemis, deflexi, ebracteati ; racemi foliis breviores. *Pedunculi* 1-1.5 mm. longi floriferi, paulo longiores sub fructu, dense pilosi pilis argenteis adpressis ; *calyx* brevis, 1-1.5 mm. longus, tubo et dentibus plus minusve aequilongis. *Corolla* calyce 3-plo longior ; vexillum dense pilosum extra, caeterae corollae partes glabrae, rubrae vel rubrescentes. *Legumen* ut plurimum monospermum, rarissime dispermum, divaricatum vel deflexum, primo pilis albidis densis ornatum, tandem subglabrum. *Semina* reniformia, olivaceo-lutea, nitentia ; cum vero bina semina adsunt, reniformia sunt sed truncata ad latera contigua.

Typus lectus a me in loco Dwarka, ad partes septentrionales Saurashtra regionis ad oras maritimas, die 16 octobris anni 1953, et positus in Blatter Herbario sub numero *Santapau* 16771 ; isotypus, *Santapau* 16784, lectus eodem die ac loco, positus etiam in Blatter Herbario ; paratypus florifer, *Santapau* 14625, lectus ad Rajkot in Saurashtra, positus est in eodem herbario.

***Indigofera articulata* Gouan var. *monosperma* var. nov.**

Very similar to the typical variety, from which it differs at once by the pods, which are 1-seeded, very rarely 2-seeded. An erect *undershrub*, more or less greyish with minute adpressed hairs, the stems and branches subterete or angular. *Leaves* 7-10 cm. long, the stipules minute and persistent. *Leaflets* 7-9, opposite, obovate or suborbicular, rounded or slightly emarginate at the apex, acute or cuneate at the base (the petiolules up to 2 mm. long), glabrous above, more or less hairy with white adpressed hairs beneath, all the leaflets subequal, or at times



TEXT-FIG. 2, *Indigofera articulata* var. *monosperma* Sant.

the terminal one slightly larger. *Flowers* axillary, racemose, up to 50 in a single raceme, deflexed, ebracteate; the racemes shorter than the leaves. *Peduncles* 1–1.5 mm. long when in flower, slightly longer in fruit, densely hairy with adpressed white hairs. *Calyx* short, 1–1.5 mm. long, the tube and teeth about equal in length. The *corolla* about three times as long as the calyx; standard densely hairy on the back, all the other parts of the corolla glabrous and red or reddish. *Pod* generally one-seeded, very rarely 2-seeded on the same plant, at first densely hairy with white adpressed hairs, at length glabrous or nearly so, divaricate or deflexed. *Seeds* reniform, olive green, shining; when there are two seeds, the adjacent sides are truncate.

The type of this variety was collected at Dwarka near the sea shore on the 16th of October 1953 and is kept in Blatter Herbarium. This is a very common shrub found all over Saurashtra. As a rule it grows erect, but occasionally, particularly near the sea or when subjected to damage, it may grow more or less prostrate, forming thin cushions. The branches of this plant are sometimes used, in the districts where it is common, as 'tooth brushes' in place of the more usual *Babul* or *Nim* trees, which are rare in the same districts. In our Botanical Survey of Saurashtra, this plant has been seen in most of the drier districts of the province, roughly all along that part of Saurashtra that lies north of Rajkot. The plant has given us much trouble, for it has been a difficult one for identification. It is distinctly a new form not previously recorded in our floras.

***Ranunculus hirtellus* Royle var. *minor* Sant. & Banerji, var. nov.**

*Ranunculo hirtello* Royle similis multis in notis, sed varietas nova a typica specie differt praesertim magnitudine multo minore, cum tota planta vix 4–6 cm. attingat; petioli varietatis ad apicem dense pilosi. Typus varietatis lectus ad Popkegola in Nepalia orientali ad 3360 m altit. a M. L. Banerji die 20 maii anni 1953, et positus in herbario Banerji sub numero *Banerji* 798.

This is a clear variety, at once distinguished from the typical plant on account of its highly reduced size, which usually does not go beyond 4–6 cm. In the Eastern Himalayas this plant seems to be widely distributed, for we have seen numerous specimens in the herbaria of Calcutta and Dehra Dun showing the typical reduction and other characters of this new variety.

***Androsace croftii* Watt var. *scaposa* Sant. & Banerji, var. nov.**

Varietas haec accedit ad typicam varietatem aspectu generali, ab ea tamen differt praesertim longitudine pedunculorum, quae est petiolis duplo saltem, saepe triplo vel ultra longior. Flores purpurascens. Typus varietatis lectus a M. L. Banerji in itinere a Thos ad Bhitri in Nepalia orientali ad altit. 2750 m die 8 mensis maii anni 1952, et positus in Herbario Blatteri in urbe Bombay sub numero *Banerji* 686; paratypus, *Banerji* 256, lectus ad eodem in itinere a Patala ad Phaplu in Nepalia orientali, ad 2710 m altit. et positus in herbario Banerji in Meerut, in India.

This new variety is quite distinct from the typical plant at first sight, mainly on account of the length of the floral scapes; in the typical plant they are just about as long as the leaves or slightly longer or at times shorter; in the new variety the scapes are at least twice, often thrice as long as the leaves, or even longer.

***Zeuxine gracilis* (Breda) Bl. Fl. Jav. N.S. 56, t. 18, f. 2, & t. 23 D, 1858; J. J. Smith, Fl. Buitenz. 6: 110, 1905 & f. 78, 1908; Holttum, Rev. Fl. Malay, 1: 134, f. 22, 1953.**

*Psychechilos gracile* Breda, Gen. Sp. Orch. t. 9, 1827.

*Monochilus affine* Lindl. Gen. Sp. Orch. 487, 1898.

*Zeuxine affinis* Hook. f. Fl. Brit. India 6 : 108, 1890 ; King et Pantling in Ann. Roy. Bot. Gard. Calcutta 8 : 290, t. 387, 1898.

*Zeuxine blatteri* Fischer in Kew Bull. 1928 : 76, 1928.

The plant or plants listed under these various names have never been mentioned for Bombay previously ; they are given here as new records for North Kanara, which until the Reorganisation of States formed part of Bombay State.

We have not seen the types of these various plants ; but a careful examination of the descriptions inclines us to accept these names as synonyms, belonging to one and the same species. The descriptions fit our plants from North Kanara.

Fisher has distinguished his *Z. blatteri* from *Z. affinis* Hook. f. by its broader leaves, its narrow petiole, its glabrous sheaths and by the lip, which is saccate, fleshy, ecalcarate, the lobes of the limb being orbicular, glabrous and distant. Examination of the description and icones of *Z. affinis* shows that the leaf sheaths are not pubescent, the lip not calcarate ; in our own specimens the lobes of the limb are rather variable, from oblong, oblong-orbicular to subcuneate, and are glabrous.

In the literature we find that the combination *Z. affinis* is attributed to Benth in Benth. & Hook. Gen. Plant. 3 : 600, 1883. Benth, however, did not make the actual combination in the sense of Art. 32 of the 1956 edition of the International Code of Botanical Nomenclature ; the combination must be credited to Benth ex Hooker f. in Fl. Brit. India, loc. cit., or simply to Hooker f.

J. J. Smith, l.c., mentions that *Z. gracilis* Blume is similar to the Indian *Z. affinis* ; but the words of R. Holttum, l.c., deserve attention : "Whether the Malayan plants are quite identical with *Z. gracilis* from Java, or with *Z. affinis* from India, is not certain. There is much variation in this group of Zeuxines, and the exact limitation of species is not certain without more careful observation of living plants."

We have gathered abundant living materials of this plant in North Kanara, and after careful examination we have come to the conclusion that the three species, *Z. blatteri* Fischer, *Z. gracilis* Blume, and *Z. affinis* Hook. f. are identical ; and in consequence the earliest name for the group is to be adopted, in this case *Z. gracilis* (Breda) Blume.

There are some notable variations in the colour of the lip, as noted by different authors. Smith describes the lip as pale flesh-coloured at the base, becoming whitish or pale yellowish upwards ; Holttum gives the lip of his plants to be yellowish at the base, with a white blade ; King and Pantling describe the lip as yellow, the blade is also coloured yellow in their plate ; Fischer states that the sac of the lip is orange, the limb white. Our own observations in the field are as follows : Sepals greenish with paler white or whitish apices ; petals greenish, but paler within and at the apices, occasionally pale pink ; the sac of the lip orange to orange red, the limb white or pale yellowish ; in specimens which have been preserved in formaline solution the sac of the lip has been noted to change to pale yellow ; the anther is pink.

### ***Epipogium* R. Br. Prodr. 330, 1810.**

This generic name was originally spelled by Gmelin as *Epipogum* (Fl. Sibir. 1 : 11, t. 2, f. 2, 1747) ; Index Kewensis has taken up the spelling of Gmelin. In the literature we find the following orthographic variants of the same name : *Epipogium*, *Epipogon*, *Epipogion*. In his Sp. Pl. 945, 1753, Linne reduced the plant to the genus *Satyrrium*, under the name *Satyrrium epipogium* ; R. Brown in 1810 revived the generic name *Epipogium*, possibly on account of the Linnean epithet. Gmelin's name is invalid as being pre-linnean i.e., of 1747 ; hence the name must be credited to R. Brown, 1810, and his spelling is the valid one.

***Epipogium roseum*** (D. Don) Lindl. in Journ. Linn. Soc. 1 : 177, 1857 : Holttum, Rev. Fl. Malay 1 : 106, 1953.

*Limodorum roseum* D. Don, Prodr. Fl. Nep. 30, Febr. 1825.

*Galera nutans* Blume, Bijdr. 416, f. 3, Dec. 1825.

*Epipogum nutans* Reichb. f. in Bonpland. 5 : 36, 1857 ; Hook. f. Fl. Brit. India 6 : 124, 1890 ; King et Pantling in Ann. Roy. Bot. Gard. Calcutta 8 : 253, 1898 ; Fischer, Fl. Pres. Madras 1460, 1928 ; Blatter et McCann in Journ. Bombay nat. Hist. Soc. 35 : 729, 1931.

*Podanthera pallida* Wight, Icon. t. 1759, 1852.

In our Indian floras this plant is commonly listed under the name of *E. nutans* Reichb. f.; however, as pointed out in Flora Males. (in 1, 4(5): clxxi) the publication of Blume's book dates from June 1 to Dec. 7, probably from the first week of December, 1825, whilst D. Don's *Prodromus* dates from February 1, 1825 ; Don's name, therefore, has priority over Blume's, and the only correct name for the plant is the one adopted here.

***Nervilia discolor*** (Blume) Schlechter in Bot. Jahrb. 45 : 403, 1911 ; Holttum, Rev. Fl. Malay 1 : 105, f. 16e, 1953.

*Cordyla discolor* Blume, Bijdr. 417, 1825.

*Pogonia discolor* Blume, Mus. Bot. Ludg.-Bat. 1 : 52, 1849, et Fl. Jav. 128, t 57, 1858 ; Smith, Fl. Buitenz. 6 : 54, 1905 & f. 33, 1908.

*Pogonia biflora* Wight, Icon. t. 1758, 1852 ; Hooker f. Fl. Brit. India 6 : 119, 1890.

*Nervilia biflora* (Wt.) Schlechter in Bot. Jahrb. 45 : 403, 1911 ; Fischer in Fl. Madras 1459, 1928 ; Blatter et McCann in Journ. Bombay nat. Hist. Soc. 35 : 726, 1931.

Blatter and McCann mention that *Bell 6066* and duplicates have been compared by C. E. C. Fischer with the type of *Pogonia biflora* Wt. in Kew Herb.; one of these duplicates is available in Blatter Herbarium, Bombay, and our specimens have been matched with it.

One result of our examination is that the two species *N. biflora* Schl. and *N. discolor* Schl. must be considered identical. There is but one point of difference, which is in the colour of the median band on the lip ; in *discolor* the band is said to be yellow, in *biflora* it is white or pale rose. The basic structure is identical, in spite of this slight variation in the colour.

The species is a very variable one, in respect of the general coloration of the leaves and flowers. When the plant grows in dense undergrowth under very reduced light intensity, the leaves are deep purple to nearly black in colour, and the covering hairs are purple and very stiff. As the monsoon comes near its end and the light and temperature of the forest goes up, the leaves turn brownish, often rusty brown. We found this plant in very large numbers in the undergrowth of the forest in the Dangs, and collected herbarium specimens and in addition a number of tubers for cultivation under controlled conditions. All our plants obtained from such tubers, which had been grown in an open part of the garden in Bombay, gave pure green leaves with pale green hairs. Further the same plant was collected from Bhimashankar, Poona Dist., in scrub forest ; these plants showed pure green leaves. It seems, therefore, that the intense purple colour of the plant is connected with deficient light. From our observations we are inclined to believe that the variation in colour of the flowers is also intimately connected with the intensity of the light at the spots in which the plant has been growing ; this is why the slight difference of colour mentioned above seems to us of little importance.



# HISTOLOGICAL STUDY OF THE SPECIALISED TISSUE OF THE AVIAN HEART

by TEJ SINGH, *Department of Zoology, Government College, Ajmer*

(Communicated by J. L. Bhaduri, F.N.I.)

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## ABSTRACT

1. The sinusvenosus being absent in these birds the sinuatrial node has lodged itself in the right atrial wall.

2. Specialised histological structures such as sinuatrial node, atrioventricular node and the atrioventricular bundle are located in the hearts of these birds.

3. The cephalic node e.g., sinuatrial node has the histological features different from atrioventricular node and the atrioventricular bundle. This disparity has been assigned to their location within the atrial tissue in one case and within the ventricular myocardium in another.

4. In the absence of well developed limbs of atrioventricular bundle in these birds a part of cardiac impulse for contraction would pass through the accessory muscle bands of ordinary cardiac fibres present in the right atrioventricular valve and at the junction of the atrial and the ventricular septa.

## INTRODUCTION

Purkinje (1845) observed under the serous sheath a network of grey, flat, gelatinous threads in the muscles and around the fibrous bundles of the heart of mammals. On microscopic examination he found these threads to be composed entirely of grains, which, like those of ganglia, were crowded close to each other and appeared polyhedral. Davies (1930) stated that these fibres constitute a special movement apparatus (Bewegungsapparat). Kulbs (1913) traced the subendocardial Purkinje fibres in the heart of birds and demonstrated the presence of a tract of tissue resembling the 'Bundle of His' of mammals at the atrioventricular junction. Mangold and Kato (1914) concluded that sinuatrial node is present in the bird's heart near the termination of the right precaval vein and the atrioventricular bundle with two terminal branches in the ventricular septum. The sinuatrial node was described by Keith and Flack (1907) as a mass of peculiar fibres having a few nerve cells and nerve fibres embedded in densely packed epicardial connective tissue. Keith and Mackenzie (1910) regarded this tissue to be intermediate between nerve and muscle fibres and therefore gave it a non-committal name of nodal tissue. Drenann (1927) by the naked eye dissection of the ostrich heart demonstrated that the atrioventricular bundle passes from the dorsal part of the base of atrial septum into the depth of the ventricular septum. Ohmori (1928) observed the atrioventricular connections between the atrioventricular node and the atrioventricular bundle in the heart of birds. Davies (1930) studied the anatomy and the conducting system of the heart of black swans and pigeons. Adams (1937) studied in detail the conducting system of the heart in kiwi and penguin. In spite of observation on the avian and mammalian heart the specialised tissue has so far not been well illustrated and there is scope for critical study.

## MATERIALS AND METHOD

Specimens of *Passer domesticus* and *Pycnonotus cafer* were locally procured and kept alive for a short period in cages. The birds were chloroformed and dissected to remove the hearts. The hearts were fixed in Bouin's picroformol. Both sagittal and frontal sections were cut off paraffin embedded blocks and stained in Van Gieson's picrofuchsin. The localised area in the sections were photographed directly.

## OBSERVATIONS

*Sinuatrial node*

The cardiac muscle fibres in these two small birds are arranged very compactly and are so fine that even in well differentiated stained sections the nuclei were hardly discernible under high power. The structure of sinuatrial node was therefore studied under phase contrast arrangement. In *Pycnonotus cafer* the sinuatrial node is a well defined structure lying on the antero-dorsal surface of the right atrial wall in a U-shaped cavity present between the opening of the left precaval vein and the septum atriorum. The node is lodged within the thin right atrial wall (Fig. 3). The sinuatrial node is not exactly a horseshoe shaped structure, its lower end touches the base of the 'inter-septo-valvular space' and at this end its fibres emerge out to become continuous with the cardiac fibres of the atrial wall. The node is separated from the left venous valve by 4 mm. and from the septum atriorum by 27 mm. The component fibres of the node are not Purkinje fibres although they are thinner than other cardiac fibres. The nodal fibres are arranged compactly within a fibrous membrane.

*Atrioventricular node*

In *Pycnonotus cafer* and *Passer domesticus* the atrioventricular node is present on the cephalic surface of the septum ventriculorum and the lower wall of the left atrium, posterior to the interatrial septum at the level of the aortic arches (Figs. 2,3). The atrioventricular node is enclosed in a mass of loosely connected fibres. The atrioventricular node is not a well defined structure in *Pycnonotus* but in *Passer* it is a well defined oval mass of Purkinje fibres.

*Atrioventricular Bundle (Bundle of His)*

The atrioventricular bundle in *Pycnonotus* and *Passer* lies ventral to the atrioventricular node. The atrioventricular node and the atrioventricular bundle in both these birds are connected through Purkinje fibres which are thinner than the component fibres of the node and the bundle. On examination of the serial sections the heart shows that the atrioventricular node is continuous with the atrioventricular bundle at its lower end. The atrioventricular bundle in *Passer* and *Pycnonotus* is composed of fine dense fibres not crossing each other with numerous prominent deeply stained nuclei (Fig. 5). The bundle covers the broad cephalic surface of the ventricular septum and gives tubular extensions on either side.

*Accessory atrioventricular muscle bands (Bundle of Kent)*

The atria and ventricle in the vertebrate heart are separated by the intervening fibrous tissue which in sections looks like transparent film taking deep red stain. In the serial longitudinal sections of the heart of *Pycnonotus* it has been observed that at the left side of the right atrioventricular opening a prominent band of atrial fibres runs vertically downward and merges with the fibres of the ventricular septum

(Fig. 6). In addition to this, as already indicated, the atrial fibres are closely applied to ventricular fibres in the case of the right atrioventricular valve which is muscular (Fig. 1).

#### *Purkinje fibres*

Purkinje fibres are poorly distributed in the atria of both the birds. Besides Purkinje fibres the author observed a distinct band of fibres unlike ordinary cardiac muscle fibres, which took deeper stain. Such a band of specialised fibres was located exactly at a place where the atrioventricular bundle was described by Davies (1930) in the heart of birds, and by His (1893) in the heart of mammals. Serial sections have revealed concentrations of Purkinje fibres on the wall of the aortic arch (Fig. 7).

### DISCUSSION

Keith and Mackenzie (1910) and Mackenzie and Robertson (1910) stated that the atrioventricular bundle, the atrioventricular node and the sinuatrial node are absent in birds. Kulbs (1913) was also not definite about the presence of the atrioventricular bundle in birds though he observed a tract of the tissue resembling the 'Bundle of His' of the mammals at the atrioventricular junction of the heart of birds. In the heart of *Pycnonotus* and *Passer* histologically specialised structures in the form of sinuatrial node, atrioventricular node and atrioventricular bundle are present.

The component fibres of the sinuatrial node and the atrioventricular node reported here are identical. Their structural differences with the atrioventricular bundle can be assigned to their atrial and ventricular origins. In the case of the two birds studied the atrial muscle is altogether different from ventricular muscle. The atrial muscle fibres are finer and thinner and thus the cephalic node (sinuatrial) is in less compromise to the septal nodes and bundles.

The atrioventricular node has been described in many avian and mammalian hearts by Tawara (1906). In avian heart it was described by Mackenzie and Robertson (1910) and Davies (1930). Ohmori (1928) described atrioventricular node in the heart of birds at the caudal end of interatrial septum. Adams (1937) described the atrioventricular node in heart of kiwi and penguin between the lowest part of the septal wall of the right atrium and the left ventricle, and embedded in the dense fibrous tissue of the 'trigonum fibrosum'. In *Passer* and *Pycnonotus* the atrioventricular node lies in the cephalic portion of septum ventriculorum and below the wall of atrium sinistrum. At this point the left atrium and septum ventriculorum are separated by a transparent tissue of loosely connected fibres, which is not a dense fibrous tissue like the trigonum fibrosum. Davies (1930) recognised an upper and lower pole in the atrioventricular node in black swans and pigeons, while Adams (1937) observed no distinction of this type in kiwi and penguins. In *Passer* the atrioventricular node is a compact, well defined structure but does not show an upper and a lower pole.

Davies (1930) observed that the atrioventricular bundle in the avian heart runs into the depth of the septum ventriculorum lying between its right and left surfaces, and divides into a right and left limb. In *Passer* and *Pycnonotus* the atrioventricular bundle gives short tubular extensions on either side, which run parallel to the surface of the ventricular septum.

Kent (1913) while making a detailed study of the heart of various mammals reported that the mammalian heart does not show a break in the muscular continuity between the atria and the ventricles and observed numerous atrioventricular muscular connections between the atria and the ventricles. He also pointed out that the idea of a single atrioventricular connection was erroneous. Since then much controversy has raged over the presence and disposition of accessory



FIG. 1.



FIG. 2.

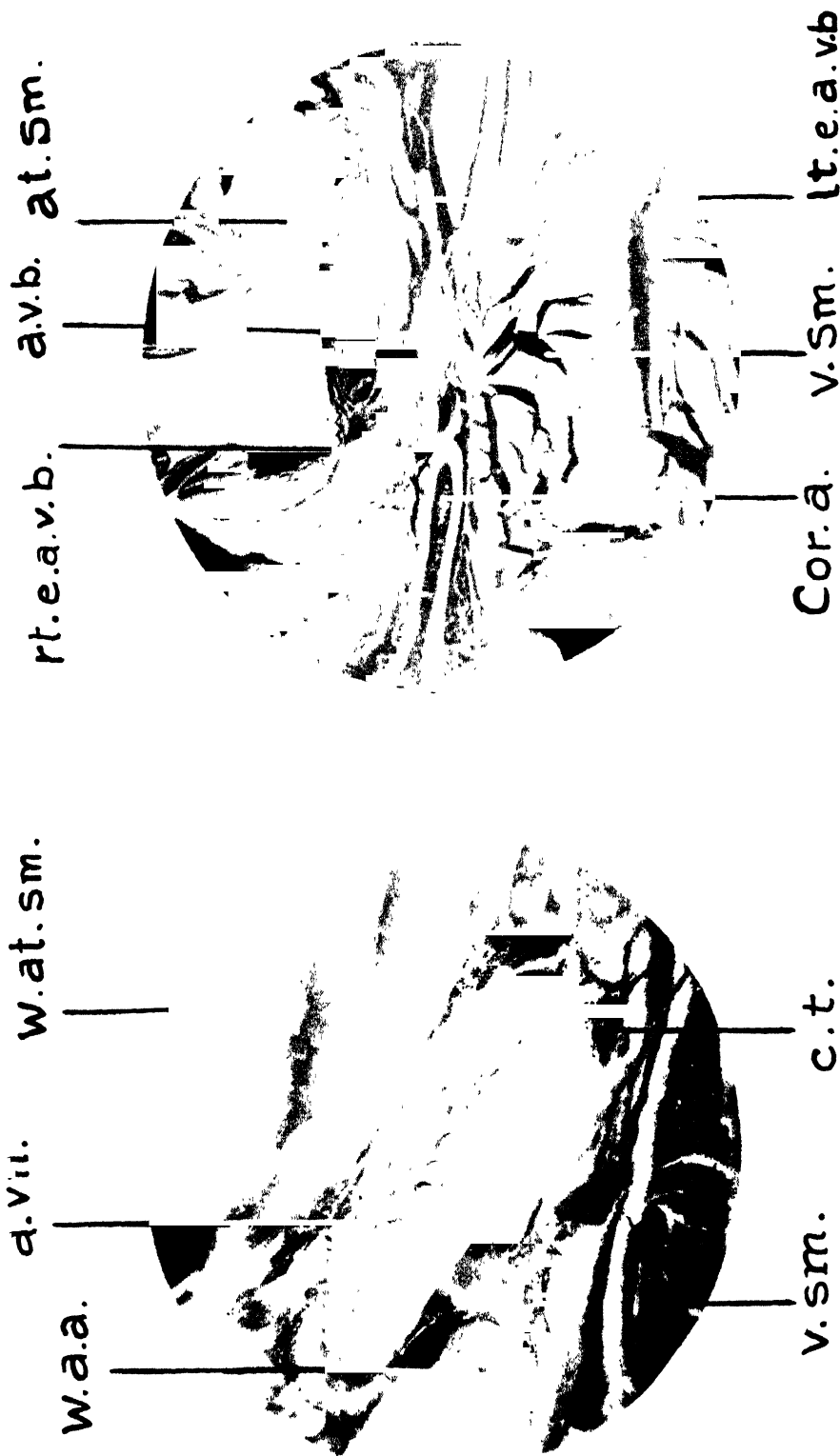


FIG. 4.

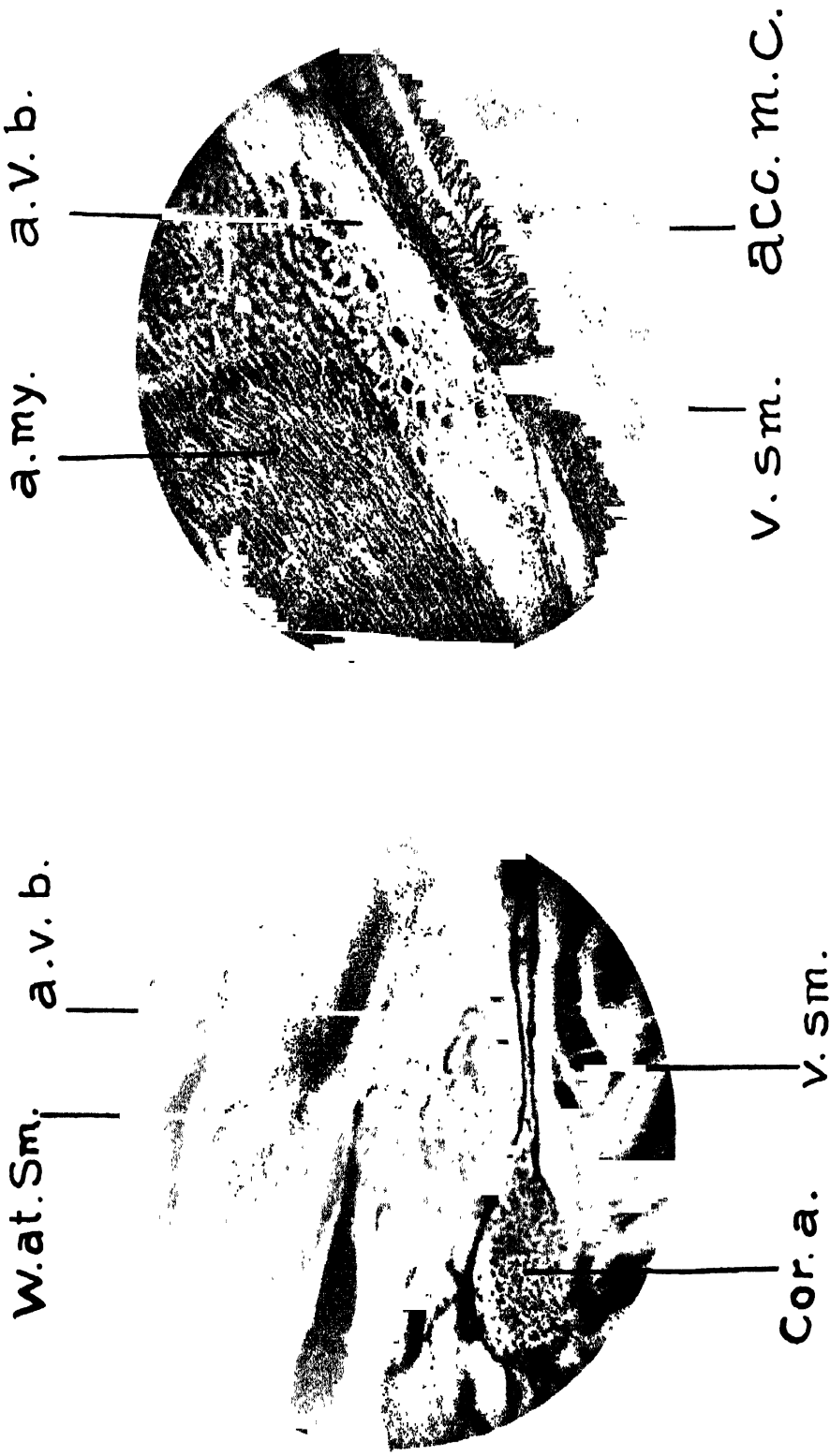


FIG. 6.

FIG. 5.



FIG. 7.

atrioventricular muscular connections. The birds lead a very active life and it has a bearing on their rapid rate of heart beat. To maintain rapid conduction of cardiac impulse the presence of well developed conducting system which provides close continuity between atria and ventricles is necessary. The histologically specialised structure e.g., sinuatrial node, atrioventricular node and the atrioventricular bundle in the case of these two birds studied are well defined. The probable path which the cardiac stimulus of contraction may take for being conducted from the sinuatrial node to the ventricle has yet to be traced.

#### ACKNOWLEDGEMENTS

The author is grateful to Dr. P. N. Mathur, Head of the Zoology Department for guidance and to the Principal, Government College, Ajmer for allowing him to conduct the present investigations.

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# EXPLANATION OF PLATES IX, X, XI, XII

(The figures are photomicrographs of localised area)

- Fig. 1. Longitudinal section through the heart of *Passer* showing right atrioventricular Valve '× 130'.  
Fig. 2. Longitudinal section through the heart of *Pycnonotus* showing atrioventricular node '× 495'.  
Fig. 3. Longitudinal section through the heart of *Passer* showing atrioventricular node '× 440'.  
Fig. 4. Longitudinal section through the heart of *Passer* showing atrioventricular bundle '× 100'.  
Fig. 5. Atrioventricular bundle under high power '× 462'.  
Fig. 6. Longitudinal section through the heart of *Pycnonotus* showing atrioventricular bundle and accessory atrioventricular muscular connection '× 490'.  
Fig. 7. Longitudinal section through the heart of *Passer* showing rings of Purkinje fibres in the wall of aortic arch '× 150'.

## ABBREVIATIONS

|             |   |
|-------------|---|
| a.a.        | Aortic arch.                                |
| a.my.       | Atrial Myocardium.                          |
| a.v.b.      | atrio-ventricular bundle.                   |
| a.v.n.      | atrioventricular node.                      |
| at.dm.      | atrium dextrum.                             |
| at.sm.      | atrium sinistrum.                           |
| acc.m.c.    | accessory muscular connections.             |
| cor.a.      | coronary artery.                            |
| c.t.        | cardiac tissue.                             |
| Lt.e.a.v.b. | Left extension of atrioventricular bundle.  |
| P.f.        | Purkinje fibres.                            |
| rt.e.a.v.b. | Right extension of atrioventricular bundle. |
| rt.v.c.     | right ventricular cavity.                   |
| rt.a.v.v.   | right atrioventricular valve.               |
| v.sm.       | septum ventriculorum.                       |
| v.my.       | ventricular myocardium.                     |
| w.at.sm.    | wall of atrium sinistrum.                   |
| w.at.dm.    | wall of atrium dextrum.                     |
| w.a.a.      | wall of aortic arch.                        |

## THE STUDY OF DRY SCRUB VEGETATION UNDER FOREST MANAGEMENT AT DHOND, POONA

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(Communicated by R. Misra, F.N.I.)

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Intense and continuous biotic interference in the Poona district has destroyed vast stretches of tree vegetation replacing it with scrubby growth or at places to stages of even poorer vegetation where only grasses grow in autumn. These degraded stretches of vegetation are still subject to continuous or even increasing pressure of grazing and are, therefore, fast heading towards denudation and destruction. If patches of such areas can be closed from grazing and other biotic interference, they can develop into dense scrub jungles where abundance of dicotyledonous plants along with grasses will serve to stabilise soil and in the conservation of water, soil and plant wealth.

During our survey of the vegetation of the Poona district we came across a large piece of land, which has been recently protected by forest department from the biotic interferences.

This land is situated on the left bank of river Bhima at Dhond ( $18^{\circ}32' N$ ;  $74^{\circ}40' E$ ), 50 miles east of Poona. It is two miles from Dhond Station between the villages of Gar and Kauthe and has an average elevation of 500 meters above sea level.

Topographically, the area is a plain country formed of traprock, greatly weathered to form a dry, coarse, sticky, hard soil on the top of which there is a layer of alluvial clay and sand deposited by river Bhima (Fig. 1).

It is situated within the flood level of the river and appears to get flooded during rains. It slopes gently towards the river and is cut up by shallow or deep gullies, some of which are even 3-4 meters deep. Along the gullies the soil of black colour is compacted.

The river Bhima in recent years seems to have been cutting its right bank and receding from its left bank on which this forest is situated. The left bank therefore has broad strip of alluvial sands and the right bank has sharply and variously cut basaltic rocks.

The area studied has been closed to grazing for the last seven years and planting of *Acacia* species has been taken up by the State Forest Department. (It is named Blank Block Nos. 9 to 13). Plantation of *Acacia* has been done in rows at about 6 meters apart. Apart from this, there does not seem to be any biotic interference at present.

The vegetation of this area is, therefore, natural and is important in the respect that it gives an indication of the succession that can come in such over-grazed areas after they are protected from interference.

The vegetation was studied in quadrats of one meter square by the method suggested by Misra and Puri (1956). In one study the quadrats were taken at random and in another two transects were laid one in north-south direction and the other in east-west direction. The shrubs and tall herbs were counted and their actual number recorded, but the ground flora species of prostrate herbs or grasses were recorded as 'abundant', 'frequent' or 'rare'. The symbols used denote the following features :

Abundant :—More than five plants in a quadrat or forming a conspicuous covering in the quadrat.

Frequent :—Plants more than three in a quadrat and their presence is conveniently noticeable.

Rare :—Less than three plants in a quadrat, or presence not noticeable.

A summary of the data is presented in Table I.

TABLE I

*Dry scrub vegetation under the forest management*

|                    |   |
|--------------------|---|
| Area :             | Garkautho Blank Block Nos. 9-13.  |
| Rock and Geology : | Greatly weathered trap basaltic rock.   |
| Soil :             | Soil is formed of weathered basaltic rock on which alluvial clay and sand is deposited. Soil dry, coarse, sticky and black in colour. |
| Biota :            | The area has been recently closed and is now practically well protected from lopping, felling or grazing.                             |

| Particulars of quadrats                  | During September,<br>40 quadrats of one<br>meter square taken<br>at random<br>in % | During November,<br>11 quadrats of one<br>meter square laid<br>in a transect<br>running east-west<br>in % | During November,<br>12 quadrats of one<br>meter square laid<br>in a transect<br>running north-south<br>in % |
|--|--|---|---|
| <b>Shrubs :—</b>                         |  |   |   |
| <i>Abutilon muticum</i> G.Don.           | 10   | 18  | 16  |
| <i>Acacia arabica</i> Willd.             | 38   | 27  | 85  |
| <i>Acacia</i> sp.                        | 10   | —   | —   |
| <i>Calotropis procera</i> Br.            | —  | 18  | 10  |
| <i>Capparis decidua</i> Pax.             | 5  | —   | —   |
| <i>Corchorus trilocularis</i> L.         | 68   | 90  | 100   |
| <i>Goniocaulon glabrum</i> Cass.         | —  | 81  | 66  |
| <b>Herbs :—</b>                          |  |   |   |
| <i>Acalypha malabarica</i> Muell.        | 35   | 9   | 8   |
| <i>Achyranthes aspera</i> Linn.          | —  | 36  | 40  |
| <i>Alysicarpus longifolius</i> W. & A.   | 13   | 18  | 8   |
| <i>Aristida funiculata</i> Trin. & Rupr. | —  | 45  | 28  |
| <i>Brachiaria isachne</i> Stapf.         | 53   | 18  | —   |
| <i>Chloris virgata</i> Sw.               | —  | 9   | —   |
| <i>Commelina forskalii</i> Vahl.         | 28   | —   | —   |
| <i>Digera arvensis</i> Forsk.            | 13   | —   | —   |
| <i>Dinebra retroflexa</i> Panzor.        | 5  | —   | —   |
| <i>Euphorbia hirta</i> Linn.             | —  | 9   | 8   |
| <i>Guizotia abyssinica</i> Cass.         | 38   | —   | —   |
| <i>Indigofera cordifolia</i> Roth.       | 60   | —   | —   |
| <i>Indigofera linifolia</i> Retz.        | 10   | 9   | —   |
| <i>Indigofera</i>                        | 18   | —   | —   |
| <i>Isachne</i>                           | —  | 63  | 50  |
| <i>Justicia quinqueangularis</i> Koen.   | 60   | 90  | 75  |
| <i>Leucas urticaefolia</i> Br.           | 8  | —   | —   |
| <i>Merremia emarginata</i> Hall.f.       | 60   | 54  | 85  |

TABLE I—Contd.

| Particulars of quadrats                  | During September,<br>40 quadrats of one<br>meter square taken<br>at random<br>in % | During November,<br>11 quadrats of one<br>meter square laid<br>in a transect<br>running east-west<br>in % | During November,<br>12 quadrats of one<br>meter square laid<br>in a transect<br>running north-south<br>in % |
|--|--|---|---|
| <i>Nazia racemosa</i> Ktze.              | 8  | —   | —   |
| <i>Paspalidium flavidum</i> Camus.       | 10   | —   | —   |
| <i>Phyllanthus maderaspatensis</i> Linn. | 23   | 72  | 35  |
| <i>Rhynchosia minima</i> DC.             | 53   | 9   | 35  |
| <i>Ruellia patula</i> Jacq.              | 15   | —   | 8   |
| <i>Rungia elegans</i> D. & G.            | —  | —   | 50  |
| <i>Solanum nigrum</i> Linn.              | —  | —   | 8   |
| <i>Sporobolus</i> sp.                    | 18   | 18  | 8   |
| <i>Tribulus terrestris</i> Linn.         | 33   | —   | —   |
| <i>Tridax procumbens</i> Linn.           | 8  | 18  | 18  |
| <i>Tripogon jacquemontii</i> Stapf.      | 10   | —   | —   |
| <i>Triumfetta rotundifolia</i> Lamk.     | 8  | —   | —   |
| <i>Urena lobata</i> Linn.                | —  | —   | 8   |
| <i>Urochloa helopus</i> Stapf.           | 15   | —   | —   |

The community is of low stature in which only a few plants grow above one meter high. The planted *Acacia arabica* shrubs sometimes grow upto 2 or 2.5 meters high. The general physiognomy of the vegetation is, however, scrubby. The plants do not have on their branches any mosses or lichens or any epiphytic ferns. Climbers are also absent emphasizing the dry nature of the locality.

The tallest plants in photograph 1 are of *Acacia arabica*. They are of different ages, varying from three to six years. *Goniocaulon glabrum*, *Achyranthes aspera* and *Abutilon* sp. are the commonest plants in the next height group i.e., 0.75 meters to 2 meters. *Achyranthes aspera*, *Goniocaulon glabrum*, and *Rungia elegans* form a mixed community. *Goniocaulon glabrum* is a tall bushy plant with light purple glaucous inflorescence and is abundant in the whole area (Fig. 2). *Abutilon muticum* is a shrub with yellow or orange flowers and is 1-1.5 meters tall. The plant is densely pubescent and has large leaves.

In the next height group i.e., between 0.5 meters and 1 meter, the following plants are common :—

*Corchorus trilocularis*, *Calotropis procera*, *Urena lobata*, *Triumfetta rotundifolia*, *Chloris barbata*. Of these *Corchorus trilocularis* is the most abundant. *Hibiscus punctatus* is also in this height group, but incidentally did not fall in any of the quadrats studied.

Occurrence of *Hibiscus punctatus* is interesting. The distribution of *Hibiscus punctatus* has been recorded by Cooke (1908) as "Gujarat: Broach collectorate (rare), Dalzell and Gibson; Surat, Dalzell! Sind: Stocks; Karachi, Woodrow! Cooke! Jemadar ka Landa, near Karachi, Stocks!"

It has not been recorded by Razi (1952) in his account of vegetation of Poona District. It appears that the plant is spreading eastwards and is a new record for this area.

The next class of plants is between height 0.1 meter and 0.5 meter. To this class belong the following plants :

*Justicia quinqueangularis*, *Rungia elegans*, *Acalypha malabarica*, *Tridax procumbens*, *Phyllanthus maderaspatensis*, *Commelina forskalii*, *Brachiaria isachne*, *Sporobolus* species, *Eclipta alba*, *Flaveria repanda*.

Among these *Justicia quinqueangularis* and *Rungia elegans* are a conspicuous feature of the vegetation. Fig. 3 shows a community of *Rungia elegans* which is more common in the river side belt between the closed and grazed areas and is frequently present on slightly raised mounds of soil. *Flaveria repanda* forms dense colonies here and there.

The last class is of plants which are prostrate or decumbent. To this class belong the following :

*Merremia emarginata*, *Phaseolus trilobatus*, *Rhynchosia minima*, *Aristida adscensionis*, *Aristida funiculata*, *Brachiaria isachne*, *Ruellia patula*, *Indigofera linifolia*, *Alternanthera echinatus*, *Indigofera cordifolia*.

Sometimes almost a pure community is seen formed of *Merremia emarginata* in the centre with *Corchorus trilocularis*, *Goniocaulon glabrum* and *Urena lobata*.

The spreading and rooting branches of *Merremia emarginata* form dense covering on the ground almost at every alternate step and are a very remarkable feature in the ground vegetation.

Fig. 4 shows a profusely branched and spreading colony of *Aristida funiculata* amidst *Merremia* and *Justicia*.

There are about ten species of grasses in the area of which *Brachiaria isachne* is the most abundant. Other more common ones are *Urochloa helopus*, *Aristida adscensionis*, *Aristida funiculata*, *Tripogon jacquemontii*, *Nazia recemosa* and *Sporobolus diander*. Since most of the grasses are small in size they are not conspicuous and are hidden in clumps of *Corchorus*, *Achyranthes*, *Flaveria* and *Abutilon*. The general appearance of the forest is not of grassland but of scrub jungle.

The following plants were also collected from this area. These incidentally did not fall within any of the quadrats studied :—*Arundinella gigantea* Dalz., *Atylosia sericca* Benth., *Caesulia axillaris* Roxb., *Capparis zeylanica* Linn., *Cardiospermum halicacabum* Linn., *Chloris montana* Roxb., *Crotalaria retusa* Linn., *Cynodon dactylon* Pers., *Desmodium diffusum* DC., *Dichanthium annulatum* Stapf., *Dicliptera roxburghiana* Nees, *Eragrostis ciliaris* Link., *Eragrostis gangetica* Steud., *Flaveria repanda* Lag., *Launea nudicaulis* Linn., *Phaseolus trilobatus* Ait., *Polygala chinensis* Linn., *Salvia plebeia* Br., *Sesbania aculeata* Poir., *Urochloa reptans* Stapf., *Uvaria lagopoides*, *Verbascum coromandelianus* Ktze, *Zizyphus jujuba* Lam.

It is significant that many of the dicotyledonous species are prostrate or decumbent herbs such as *Merremia emarginata*, *Euphorbia* sp., *Tribulus terrestris* and *Rhynchosia minima*. This feature of the vegetation also indicates the dry nature of the soil and intense grazing in the past. It will be interesting to note in the course of time, how the frequency of prostrate and erect herbs changes under conditions of protection to the forest.

The area is almost devoid of any tree seedlings although trees of *Acacia*, *Capparis decidua* and *Capparis zeylanica* grow in the neighbourhood of this closed forest. Just adjacent to the closed forest are fields open to grazing. They not only lack in any tree vegetation but the shrubs also are represented by merely two or three species such as *Cassia tora* Linn., *Zizyphus jujuba* Lam. and *Capparis decidua* Pax. The few grasses and dicotyledonous plants growing in this open area are interesting as they are quite different from those growing in the closed area. Plants of *Eclipta alba*, *Setaria intermedia*, *Paspalidium flavidum* and *Digitaria marginata* are common.

The *Acacia* forest between the reserve plot and the river bank has only small *Acacia* and *Capparis* trees and shrubs but being open to grazing there are no other shrubs or any noteworthy ground flora. Fig. 5 shows scattered small trees of *Acacia arabica* and *Capparis decidua*. The ground is barren except for a few



Fig. 1. A general view of the Garkautha forest from Dhond town. The river Bhima is seen with islands of hard basaltic rock in the centre.

Fig. 2. A community of *Goniocaulon glabrum*.

Fig. 3. A pure community of *Rungia elegans*.



Fig. 4. A profusely spreading colony of *Aristida funiculata*.

Fig. 5. A view of the open *Acacia* forest. The ground is devoid of any noteworthy vegetation except browsed stumps of grasses, *Corchorus* etc.

Fig. 6. A bushy branched shrub of *Capparis decidua* in flower.

scattered distant patches of browsed shrubs and small stumps of *Corchorus*, *Xanthium* and grasses. Fig. 6 is of a bush of *Capparis decidua* bearing orange flowers.

It is not possible at present to give any successional trend in the development of vegetation under closed or open conditions. The observations are of interest, because they describe the two contrasting types of vegetation under open and closed conditions of forest.

#### ACKNOWLEDGMENTS

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# A NEW METHOD FOR THE CLASSIFICATION OF THE CLIMATES OF THE ARID AND SEMI-ARID REGIONS\*

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(Communicated by F. R. Bharucha, F.N.I.)

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## ABSTRACT

After pointing out the limitations of the existing systems of climatic classification a new system based on the Effective Growth Index has been evolved. This system is applied to classify the arid and semi-arid regions of India and vicinity by using climatic normals, such as temperature and precipitation of sixty stations situated in and around the arid and semi-arid regions. The computed Effective Growth Indices were plotted on a map and through the study of vegetation, soil and landscape it was found that places having the Effective Growth Indices between 0 and 20 have arid climate while those with Effective Growth Indices between 20 and 40 belong to the semi-arid one.

The purpose of climatic classification is to characterise climatic regions in terms of those principal elements, such as temperature and precipitation, which are the most decisive in the formation of the vegetation and soil groups of the earth's surface. It attempts to develop the similarities which the climatic elements bear to each other and their relation to their principal causes, the effect of latitude, of atmospheric and oceanic circulation, in opposition to geographical accidents.

Climatic classifications owe their origin to phytogeographical descriptions though in recent years there has been some controversy in defining this subject and its relation to meteorology, geography and statistics. The physiognomic descriptions of plant associations are related to the first descriptive maps of the distribution of temperature and rainfall, and in this connection we should cite valuable contribution of de Candolle (1875), Grisebach (1875), and Schimper (1903), that include the analysis of the diverse plant types of the world in relation to the principal climatic elements which determine them.

Köppen, a St. Petersburg-trained biologist, and a contemporary of the phytogeographers mentioned above, was the first to attempt a rational classification of the major climatic groups, taking as a basis the phyto-geographical manifestations which they determined and adopting a terminology and symbolism similar to those used by de Candolle. His first classification appeared in 1900 and it has been repeatedly changed both by himself and others. It finally appeared in the *Handbuch der Klimatologie* published by him in 1936.

Köppen's system was widely accepted by several authors but each one of them admitted its obvious defects. Köppen, in his work, had recognised the importance of evaporation to distinguish between humid and dry climates but being unable to measure it, he developed as his indices relations between precipitation and temperature weighing both in such a manner that the resulting indices would correspond to the boundaries between the actual vegetation types. Since then, many authors have directed their attention to solve this problem, with an

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\* Paper read at the International Geographical Seminar in the Session on "Arid and Semi-arid Zones" held at Aligarh University in January, 1956.

idea to improve upon the primary concept laid down by Köppen. Notable among such formulae being Mayer's (1926) "Total Precipitation to mean Saturation Deficit", Vazquez's (1933) "Phyto-climatic Index", Rosenkranz's (1936) "Index of Oceanicity" and Davidson's (1934) "Precipitation-Evaporation ratio".

The "Precipitation-Evaporation ratio" though very attractive to use, could not be computed for all regions due to lack of necessary data and hence demanded some alternative, and the formulae put forward usually took into account precipitation and temperature or these in some of their functional forms. Among such formulae mention may be made of Lang's (1915)  $P/T$ , Albert's (1928) "Reduced Rain Factor," de Martonne's "Index of Aridity".

Thornthwaite (1931), in his first attempt, rejected both Köppen's and de Martonne's classifications and offered a new one obtained by summing the individual month's "Temperature Efficiency" and "Precipitation Effectiveness". This "Precipitation Effectiveness" is a function of rainfall and temperature evolved to fit the loss of water from open pans run by U.S. Weather Bureau at 21 stations in the western part of the country and is applicable to only those figures, as has been proved by Bharucha and Shanbhag (1957). His formula when solved for evaporation shows that the evaporation varies directly as the approximate temperature and has a small inverse dependence on precipitation. This relation is true to some extent (if we do not reach the limiting condition) where unlimited supply of water is available for evaporation, but in case of soil surfaces in nature it would be contrary to our experience to say that the evaporation increases as the amount of water in the soil decreases.

There are many expressions with the aid of which evaporation from open water surfaces can be predicted though all of them are to some extent empirical, but they make at least some attempt at physical realities. One such formula is by Rowher (1931) which has been thoroughly tested both in the laboratory and outside, the second is by Thornthwaite himself derived in collaboration with Holzman (1942) and the third is by Penman (1948). Evaporation data obtained from any one of these formulae can be used in conjunction with precipitation to classify the climates instead of using the expression as laid down by Thornthwaite which gives results quite contrary to our experience.

It may, however, be doubted whether evaporation from pans or large bodies of water, is in any way representative of the evaporation from soils covered with vegetation. In nature there are two processes viz., (i) the evaporation from the soil and (2) the transpiration from the plants which are active in transporting the precipitated water back into the atmosphere. These processes are governed mainly by the sun and the sky radiation, the atmosphere immediately above the ground in which actual vapour pressure is less than the saturation vapour pressure, the wind movements which remove the excess of water in the air above the evaporating surface and thus prevent the saturation point being reached, and finally the temperature of the air. The last, viz., the air temperature plays an important rôle in at least two ways:—(i) it controls the temperature of the water through thermal convection and thus acts as a source or sink for heat energy depending upon whether the air is warmer or colder than water; and (ii) since it controls the temperature of water it partially determines the vapour pressure deficit.

Evaporation is also controlled by the nature of the container through its response or relation to the meteorological factors mentioned above. Thus, for example, the sun and the sky radiation falling on the soil is retained in a thin layer near the surface since soil is opaque to light and thus will cause a large rise in the soil surface temperature as opposed to that of water which is translucent to light. Hence evaporation from saturated soil will be greater than that from a large mass of water under similar meteorological conditions. This is true for a wet soil surface, but as soon as the surface becomes dry other factors come into operation. At this stage evaporation does not take place from the interface of soil and air, but a few

millimeters below. Hence the water molecules have to diffuse through the dry soil which acts as a "potential barrier" before being caught and blown away by the prevailing wind. This potential barrier which increases the path of molecular diffusion, reduces the rate of evaporation. Secondly, heat conduction from the surface into the deeper layer of the soil becomes smaller as the pores are filled with air whose thermal conductivity is smaller than that of water.

Transpiration which is more powerful than evaporation in reducing the available water is also governed by the same meteorological factors which bring about evaporation. Besides these it depends upon the physical and biological factors of the soil and plant respectively. The meteorological factors which bring about transpiration are considerably altered by the location, colour and orientation of trees and their leaves. The shaded leaves will transpire less than the unshaded ones because of the difference in the available sun and sky radiation. It is an established fact that the wind increases with height in the layers nearest the ground with the result that transpiration from short grasses will be less than that from isolated trees, other things being equal. The colour of the leaves which decides the albedo will in turn govern the rate of transpiration. Orientation of the leaves with respect to the incident beam of light determines the amount of absorbed energy and this in its turn fixes the rate of transpiration. There are numerous other examples to show that the rate of transpiration will be affected by the modification of the meteorological factors through the transpiring agent, the plant. The physical and biological factors which control the rate and amount of transpiration are too complicated to be mentioned here, but for a detailed account reference may be made to the present author's work (1957).

A short description of the processes of evaporation and transpiration given above makes it clear that both these phenomena depend upon several agents and they cannot be equal or in any way comparable to the water loss from the evaporating pans or atmometers. Furthermore, in the case of the evaporating pans or atmometers the substance to be evaporated, viz., water, is unlimited and the rate of evaporation is solely governed by the evaporating agents as modified by the nature of the container. In nature, however, such condition of unlimited supply of water occurs only during the wet season and even during this season the supply of water depends upon the intensity and distribution of the storm as well as on the soil, vegetation and landscape. The evaporation off an open water surface is sometimes regarded as being a measure of the evaporating power; even if this proposition is accepted, it can however be regarded as sound only for unit area of an infinite water surface. The evaporation from small pans is affected by the turbulence set up across the rims of the pans. Under such condition it is rather difficult to tell as to what is being measured by these pans. For these reasons the results or relations derived from such studies cannot be used to define the aridity or humidity of a place. Realising that his precipitation-evaporation ratio and the temperature efficiency indices can give little hint of the complexity of soil moisture relationship and that they cannot be regarded as adequate measure of precipitation efficiency, Thornthwaite (1948) proposed his second classification based on the humidity and aridity indices which is the difference between the precipitation and the "consumptive use". The term "consumptive use" which was in vogue in irrigation practices was introduced into climatology by Thornthwaite, changing its name into what is called "potential evapotranspiration". It is defined as the amount of water which would be lost from a surface completely covered with vegetation and supplied with ample water. This quantity, according to Thornthwaite but not in the opinion of Blanny (1953) who introduced the term "consumptive use", depends only on the amount of solar energy received by the surface and the resulting temperature rather than on the kind of plant. This hypothesis is quite contrary to the findings of Henrici (1946) and Blannay and Criddle (1945).

Thornthwaite's concept of P-E which may be useful in irrigation practices, becomes highly hypothetical for the classification of climates since the required condition of land completely covered with vegetation and supplied with adequate water throughout the year occurs only in a limited part of the world. Secondly, the empirical relation between evaporation and temperature obtained by him is based on the data of water requirements of crops at 12 irrigated areas in U.S.A. in latitudes ranging from 29°N to 43°N and is subjected to the same criticism as was directed to his first. Thirdly, though he says that the P-E depends only on the amount of solar energy, his formula for obtaining the monthly or daily P-E is based on a correlation with the mean monthly temperature; but the mean monthly temperature is in itself not a measure of the available energy for evaporation and transpiration. Any formula which is based on temperature and empirically corrected for latitudes gives erroneous values of P-E if the temperature of a locality is largely influenced by high altitude, presence of warm and cold ocean currents and advective air. His formula, as it stands, gives high values of P-E during the winter months than what could be explained on the energy consideration. Besides these there are other shortcomings in his system of classification. They are:—(a) that the water which could be held in the soil and is made available to the plant is 4 inches for all soils; (b) that the energy needed to withdraw this amount of water is the same at all moisture contents till the wilting point is reached; (c) that amount of water which is available for run-off is 50 per cent of the water surplus for all landscape and soils: which are not at all acceptable.

In nature transpiration and not evaporation from the soil, which stops as soon as an insulating dry cap is formed on its surface, is more powerful in removing water in unit time by acting simultaneously on the whole depth of the soil column occupied by the root zone and also removes more water in the long run since it reaches to a greater depth than what evaporation can do. Hence evaporation in nature which takes place primarily through transpiration is not really a loss to be deducted from rainfall, as is done by Thornthwaite for finding the availability of water for plant growth. In fact, the opposite is more nearly true; the natural evaporation from soil covered with plants represents almost entirely the water that has been used in growth with the result that places where there is high evaporation must have a luxuriant growth of vegetation, which fact is not brought about by Thornthwaite in any of his classification.

It has been already remarked that the P/E ratio is very attractive in the classification of the climates of the continents but the determination of the natural evaporation is very difficult. The only sound method for its determination is based on turbulence theory that was first formulated by Thornthwaite and Holzman (1942) and achieved its perfection in the hands of Pasquill (1949). Determination of this quantity based on the above method is very meagre. In the absence of such data we have to confine our attention to other more reasonable methods of classification rather than use some hypothetical quantity which has no real physical existence.

#### TEMPERATURE AND PLANT GROWTH

Temperature influences in one way or other every chemical and physical process connected with plants. Solubility of minerals, absorption of water, gases, mineral nutrients, diffusion, synthesis as well as the vital processes, such as growth and reproduction are all controlled by temperature. Since these processes are necessary for plants to get established and to survive, temperature controls to a considerable extent the distribution of plants on the earth and largely determines the flora of the different regions. Moreover, temperature delimits also the area of successful production of most agricultural crops.

Several investigators tried to find a relation between temperature and growth. All of them came to the conclusion that there is always an optimum temperature for growth, a temperature at which the growth rate is the highest. The growth rate decreases at a lower or higher temperature. This optimum temperature when the growth rate is maximum is in the vicinity of 30°C (86°F) though it is found to vary slightly with the species and length of their exposure. Lehenbaur (1914) from his investigation on the growth of maize seedling in relation to temperature found that the greatest rates of growth occur within the temperature range 29° to 32°C. Wadley (1936) from his study of Green Bugs in relation to temperature found that the rate of development was most rapid at 30°C. Similarly, there are minimum and maximum temperatures beyond which growth does not occur. These limits, though variable, are in the vicinity of 0°C for the lower value and somewhere about 40°C for the upper value.

Van't Hoff (1884) propounded the famous principle that the velocity of any chemical reaction doubles or trebles with each rise in temperature of 10°C. His equation is of the form :—

$$V = CK^t$$

where the constant  $K$  has the value 1.0718 and 1.1161 when the velocity doubles or trebles respectively for a rise of temperature of 10°C. When the growth rates as given by Lehenbaur are compared it is found that Van't Hoff's law is applicable only in a limited range of 20° to 31°C and nowhere else.

All investigations on the relation between temperature and growth have made it clear that up to 30°C there are the growth stimulating factors and beyond that the growth inhibiting factors come into operation. What these growth inhibiting factors are, is uncertain. But these relations between temperature and growth cannot be explained by the Van't Hoff's quotient which goes on increasing as the temperature increases.

A satisfactory formula for the growth index is in the form :—

$$V = \frac{pqr e^{rt}}{(e^{rt} + q)^2}$$

where  $p$ ,  $q$ ,  $r$ , are constants,  $t$  is the temperature in °C and  $e$  is the base of Napierian logarithm. In the above equation the numerator stands for growth stimulating factor while the denominator represents the growth inhibiting factor. The equation is solved in the light of the data published by Lehenbaur and the values of the constants are found out. They are :— $p = 1681.0$ ;  $q = 1118.8$ ; and  $r = 0.24$ . Using these constants a table has been prepared by the present author (1956) to enable one to compute the growth indices for any temperature in the range 0° to 50°C.

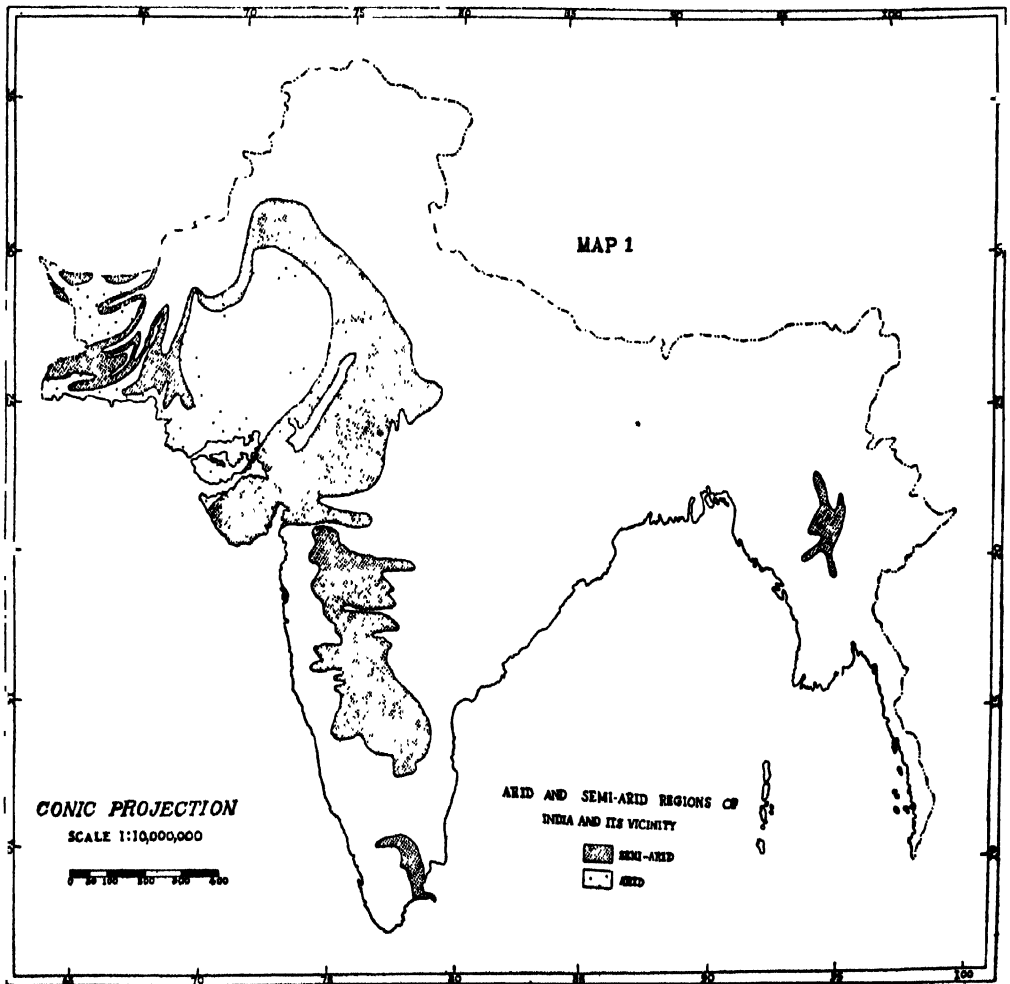
The monthly precipitation ( $P$ ) of a station when divided by the growth index corresponding to the mean monthly temperature of that station and multiplied by 100 (to save the inconvenience of fractions) gives what is called the "monthly effective growth index" and its summation for all the months of the year gives what is termed as the "Effective Growth Index" and is represented symbolically as E.G.

The formula for getting the "Effective Growth Index" is :—

$$E.G. = \sum_{n=1}^{n=12} \left\{ \frac{100(P)[e^{0.24t} + 1118.8]^2}{(1681.0)(1118.8)(0.24)(e^{0.24t})} \right\}_n$$

This formula was first of all applied to study the arid and semi-arid regions of India and vicinity. The E.G. was calculated for sixty stations in and around

our arid and semi-arid regions. These stations had temperature and precipitation records extending over a period of 30 years or more. The computed effective growth indices were plotted on a map of scale 1 : 5,000,000 and the *E.G.* isopleths were drawn. Through the study of vegetation, soil and landscape it was found that if the *E.G.* of a station falls between 0 and 20 its climate is arid, while, if it lies between 20 and 40, it is of the semi-arid type (See Map I, reduced copy of the original).



Examples of computation for five stations of the arid type and five of the semi-arid type are given in Table I.

TABLE I

| Item                             | Jan. | Feb. | Mar. | Apr.  | May   | Jun.  | July  | Aug. | Sep. | Oct. | Nov. | Dec. | Sum   | Climatic type |
|----------------------------------|------|------|------|-------|-------|-------|-------|------|------|------|------|------|-------|---------------|
| BIKANER, LAT. 28°N, LONG. 73°E.  |      |      |      |       |       |       |       |      |      |      |      |      |       |               |
| T                                | 16.7 | 18.9 | 25.0 | 31.1  | 35.0  | 35.6  | 33.3  | 31.7 | 31.1 | 28.3 | 22.2 | 16.7 | ..    | Arid.         |
| P                                | 0.31 | 0.31 | 0.20 | 0.20  | 0.71  | 1.50  | 3.11  | 3.39 | 1.50 | 0.31 | —    | 0.20 | 11.74 |               |
| G.I.                             | 18.1 | 28.7 | 78.7 | 96.0  | 64.6  | 59.1  | 80.3  | 92.6 | 96.0 | 99.6 | 53.0 | 18.1 | 784.8 |               |
| R                                | 1.7  | 1.1  | 0.2  | 0.2   | 1.1   | 2.5   | 3.9   | 3.7  | 1.6  | 0.3  | —    | 1.1  | 17.4  |               |
| DWARKA, LAT. 22°N, LONG. 69°E.   |      |      |      |       |       |       |       |      |      |      |      |      |       |               |
| T                                | 18.5 | 20.3 | 23.5 | 26.3  | 28.3  | 29.3  | 28.1  | 26.8 | 26.7 | 26.8 | 24.4 | 19.7 | ..    | Arid.         |
| P                                | 0.05 | 0.10 | 0.83 | 0.12  | 0.01  | 3.62  | 8.14  | 2.11 | 1.25 | 0.07 | 0.01 | 0.08 | 16.39 |               |
| G.I.                             | 26.5 | 37.8 | 64.9 | 89.4  | 99.6  | 100.0 | 99.0  | 92.6 | 92.0 | 92.6 | 73.1 | 33.6 | 901.1 |               |
| R                                | 0.2  | 0.3  | 1.3  | 0.1   | 0.01  | 3.62  | 8.2   | 2.3  | 1.4  | 0.8  | 0.01 | 0.2  | 18.44 |               |
| JAMNAGAR, LAT. 23°N, LONG. 70°E. |      |      |      |       |       |       |       |      |      |      |      |      |       |               |
| T                                | 18.9 | 20.6 | 25.0 | 28.3  | 30.6  | 31.1  | 29.4  | 27.8 | 27.2 | 26.2 | 24.4 | 20.0 | ..    | Arid.         |
| P                                | 0.00 | 0.20 | 0.20 | 0.00  | 0.00  | 1.89  | 7.80  | 7.01 | 2.01 | 0.00 | 0.00 | 0.08 | 19.19 |               |
| G.I.                             | 26.7 | 40.0 | 78.7 | 99.6  | 98.3  | 96.0  | 100.0 | 97.9 | 95.0 | 98.3 | 73.1 | 35.7 | 939.3 |               |
| R                                | —    | 0.5  | 0.2  | —     | —     | 2.0   | 7.80  | 7.2  | 2.1  | —    | —    | 0.2  | 20.0  |               |
| KARACHI, LAT. 25°N, LONG. 67°E.  |      |      |      |       |       |       |       |      |      |      |      |      |       |               |
| T                                | 18.3 | 20.0 | 23.3 | 26.1  | 28.9  | 30.0  | 28.9  | 27.8 | 27.2 | 26.7 | 22.8 | 19.4 | ..    | Arid.         |
| P                                | 0.51 | 0.39 | 0.31 | 0.20  | 0.08  | 0.91  | 2.91  | 1.69 | 0.39 | —    | —    | 0.20 | 7.59  |               |
| G.I.                             | 25.1 | 35.7 | 63.6 | 87.4  | 100.0 | 100.0 | 100.0 | 97.9 | 95.0 | 92.0 | 58.4 | 31.8 | 886.9 |               |
| R                                | 2.0  | 1.1  | 0.5  | 0.2   | 0.08  | 0.91  | 2.91  | 1.70 | 0.4  | —    | —    | 0.6  | 10.4  |               |
| SAKKAR, LAT. 28°C, LONG. 69°E.   |      |      |      |       |       |       |       |      |      |      |      |      |       |               |
| T                                | 14.8 | 18.6 | 23.2 | 28.8  | 34.2  | 35.7  | 34.3  | 32.8 | 32.0 | 28.1 | 22.3 | 16.9 | ..    | Arid.         |
| P                                | 0.20 | 0.28 | 0.24 | 0.12  | 0.16  | 0.16  | 0.16  | 0.10 | 0.04 | —    | —    | 0.04 | 2.5   |               |
| G.I.                             | 11.8 | 27.0 | 62.1 | 100.0 | 72.1  | 58.6  | 71.2  | 84.3 | 90.5 | 99.0 | 53.8 | 18.8 | 749.2 |               |
| R                                | 1.7  | 1.0  | 0.4  | 0.1   | 0.2   | 0.3   | 0.2   | 1.3  | —    | —    | —    | 0.2  | 5.4   |               |

TABLE I—Contd.

| Item  | Jan. | Feb. | Mar. | Apr.  | May  | Jun.  | July  | Aug.  | Sep.  | Oct.  | Nov.  | Dec. | Sum    | Climatic type |
|---|------|------|------|-------|------|-------|-------|-------|-------|-------|-------|------|--------|---------------|
| AHMADABAD, LAT. 23°N, LONG. 72°E.   |      |      |      |       |      |       |       |       |       |       |       |      |        |               |
| T   | 21.1 | 23.3 | 27.8 | 31.7  | 33.9 | 32.8  | 29.4  | 28.3  | 28.9  | 29.4  | 26.1  | 22.8 | ..     | Semi-arid.    |
| P   | —    | 0.08 | —    | —     | 0.51 | 3.78  | 12.28 | 8.19  | 4.29  | 0.51  | 0.20  | —    | 29.84  |               |
| G.I.  | 43.9 | 63.6 | 97.9 | 92.6  | 74.9 | 84.5  | 100.0 | 99.6  | 100.0 | 100.0 | 87.4  | 58.4 | 1002.8 |               |
| R   | —    | 0.13 | —    | —     | 0.70 | 4.48  | 12.28 | 8.22  | 4.29  | 0.51  | 0.23  | —    | 30.84  |               |
| AHMADNAGAR, LAT. 19°N, LONG. 74°E.  |      |      |      |       |      |       |       |       |       |       |       |      |        |               |
| T   | 20.3 | 21.7 | 27.2 | 29.4  | 30.3 | 27.8  | 25.0  | 25.6  | 25.0  | 25.0  | 20.3  | 19.7 | ..     | Semi-arid.    |
| P   | 0.20 | 0.08 | 0.20 | 0.39  | 0.91 | 5.20  | 3.78  | 2.72  | 6.50  | 2.21  | 1.10  | 0.59 | 23.88  |               |
| G.I.  | 37.8 | 48.7 | 95.0 | 100.0 | 99.3 | 97.9  | 78.7  | 83.7  | 78.7  | 78.7  | 37.8  | 33.6 | 869.9  |               |
| R   | 0.54 | 0.16 | 0.21 | 0.39  | 0.92 | 5.31  | 4.80  | 3.25  | 8.26  | 2.81  | 2.91  | 0.18 | 29.74  |               |
| MUNYWA, LAT. 22°N, LONG. 95°E.  |      |      |      |       |      |       |       |       |       |       |       |      |        |               |
| T   | 21.2 | 23.7 | 28.1 | 31.4  | 32.1 | 30.4  | 30.4  | 29.9  | 29.4  | 28.1  | 25.1  | 21.4 | ..     | Semi-arid.    |
| P   | 0.04 | 0.08 | 0.12 | 0.91  | 4.76 | 4.65  | 2.95  | 4.65  | 5.98  | 5.20  | 1.58  | 0.32 | 31.24  |               |
| G.I.  | 44.7 | 66.6 | 99.0 | 94.5  | 89.9 | 98.9  | 98.9  | 100.0 | 100.0 | 99.0  | 79.5  | 46.2 | 1017.2 |               |
| R   | 0.09 | 0.12 | 0.12 | 0.96  | 5.29 | 4.70  | 2.98  | 4.65  | 5.98  | 5.25  | 1.99  | 0.69 | 32.82  |               |
| PANBAN, LAT. 9°N, LONG. 79°E.   |      |      |      |       |      |       |       |       |       |       |       |      |        |               |
| T   | 25.9 | 26.6 | 28.2 | 29.7  | 30.2 | 29.6  | 29.1  | 28.9  | 28.9  | 28.2  | 27.1  | 26.9 | ..     | Semi-arid.    |
| P   | 2.56 | 0.87 | 0.71 | 1.85  | 0.98 | 0.16  | 0.47  | 0.59  | 1.14  | 8.54  | 11.73 | 7.60 | 37.20  |               |
| G.I.  | 86.2 | 91.3 | 99.3 | 100.0 | 99.7 | 100.0 | 100.0 | 100.0 | 100.0 | 99.3  | 94.6  | 93.2 | 1163.6 |               |
| R   | 2.97 | 0.95 | 0.72 | 1.85  | 0.98 | 0.16  | 0.47  | 0.59  | 1.14  | 8.60  | 12.37 | 8.15 | 38.95  |               |
| RAJKOT, LAT. 22°N, LONG. 71°E.  |      |      |      |       |      |       |       |       |       |       |       |      |        |               |
| T   | 19.7 | 21.4 | 25.8 | 29.7  | 32.2 | 31.7  | 28.6  | 27.5  | 27.8  | 27.8  | 24.2  | 20.6 | ..     | Semi-arid.    |
| P   | —    | 0.08 | 0.08 | —     | 0.51 | 4.41  | 10.39 | 5.59  | 3.90  | 0.59  | 0.31  | 0.08 | 25.94  |               |
| G.I.  | 33.6 | 46.2 | 85.4 | 100.0 | 89.2 | 92.6  | 100.0 | 96.6  | 97.9  | 97.9  | 71.4  | 40.0 | 950.8  |               |
| R   | —    | 0.17 | 0.09 | —     | 0.57 | 4.76  | 10.39 | 5.79  | 3.98  | 0.60  | 0.43  | 0.20 | 26.98  |               |
| Note :—T— Temperature in °C, P— Precipitation in inches, G.I.— Monthly Effective Growth Index, R— Ratio P/G. I. |      |      |      |       |      |       |       |       |       |       |       |      |        |               |

Note:—T — Temperature in °C, P — Precipitation in inches, G.I. — Monthly Effective Growth Index, R — Ratio P/G. I.



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# THE VEGETATION OF MARSHES AND SWAMPS IN THE POONA DISTRICT

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## INTRODUCTION

Although other types of vegetation have been studied in this country to some extent, the study of the marshes and swamps has not attracted much attention from botanists. There is no account of this type in Champion's survey (1938). This type is of great interest botanically, besides possessing possibilities of their being put to some economic uses. *Typha*, *Trapa*, *Nelumbo*, and several grasses that are of considerable economic use occur in moist habitat. It was, therefore, considered necessary to make a study of this type of vegetation in our normal exploration work.

There are several areas in the Poona district, which receive a heavy rainfall but on account of the porous nature of the soil, ordinarily water does not collect and, therefore, large marshy or swampy areas do not exist in this district. In some places due to seepage of water from canals or the collection of water in low places swamps are created. Some of these dry up soon after the rains, but here and there permanent marshes and swamps are met with. The vegetation of these is described in this paper. The study was made according to the method suggested by Misra and Puri (1956).

## LOCATION OF THE MARSHY AND SWAMPY HABITATS

On account of the reason explained above, marshes and swamps are not restricted to any particular areas or places. The studies were therefore conducted over a number of places scattered all over the district in different seasons.

1. *Vitthalwadi*: Vitthalwadi is a small village famous for the temple of 'Vitthal' or 'Pandurang', and it is situated at a distance of 6 miles from Poona on Poona-Sinhagad road. Near the 5th mile along the road, marshy areas are formed over the first and second terrace of the river Mutha. The marshes are formed by the seepage of water from the Khadakwasla canal running almost parallel to the road. There are marshy places along the river as well (Fig. 1). The soil is very shallow in most of the places and the marshes are restricted to small patches.

2. *Sholapur Road* (Kunjarwadi): Along the Poona-Sholapur road, near the village Kunjarwadi, there is a marsh spread over a considerably large area. The water comes mostly from the canal, but a small amount of drainage water might be coming from the nearabout fields. The soil is deep black, clayey and mixed with a small amount of sand and gravel. 11 quadrats of 1/3 meter sq. were studied from this area.

3. *Sholapur Road* (Saizpur): At the foot of a hillock, 21.6 miles from Poona along Poona-Sholapur Road is a small pool, which is surrounded by marshy vegetation. This marsh is restricted to a small area, and in all probability, it is only a seasonal marsh. 14 quadrats of 1/3 meter square were observed and recorded.

4. *Bhosari* : A fairly large swamp is met with along Bhosari lake, 5 miles from Kirkee on Poona-Nasik Road (Fig. 2). This is naturally a permanent swamp, and some parts of it form a water-logged condition. The soil is sticky, black and deep. Number of quadrats studied from this area was 10.

5. *Poona-Nasik Road* : About 4.2 miles from Kirkee, where Poona-Nasik road and the proposed railway line cross each other, one can see a dense *Typha* community growing in a swamp. The swamp is formed due to a water stream originating just nearby. 10 quadrats of 1/3 meter square were studied.

6. *Ghorwadi* : Just by the side of Ghorwadi Railway Station (about 20 miles from Poona, Bombay-Poona Railway Line), marshy type of vegetation is thriving along small pools. The soil is shallow, brownish and somewhat sticky, and the marshes dry up in summer.

7. *Talegaon* : A large and old lake is situated at Talegaon (Dabhade), on the border of which small patches of marshes are formed. 10 quadrats of 1/3 meter square were studied both at Ghorwadi and at Talegaon.

#### GENERAL DESCRIPTION OF VEGETATION

The marsh and swamp vegetation is formed of a number of communities, among which communities of *Typha*, *Asteracantha*, *Caesulia*, *Cyperus*, and grasses are prominent. In the *Typha* community, other plants are normally not present, but the grass communities are usually mixed. Communities of *Asteracantha*, *Caesulia*, *Cyperus* etc., may be pure or mixed.

The data for the communities from the various places mentioned above are summarized in Table 1, and detailed descriptions of communities are given separately.

##### 1. *Fimbristylis-Cyperus* Community

This community (Fig. 3) is formed mainly by two species of the *Cyperaceae*, namely *Fimbristylis diphylla* Vahl which occurs in 53.0 per cent of the quadrats and *Cyperus globosus* All. which has a percentage occurrence of 35.4. *Fimbristylis diphylla* Vahl is dominant both in distribution as well as in height, reaching upto 60 cms.; while the maximum height of *Cyperus globosus* All. is 50 cms. The third important element of the community is *Fimbristylis woodrowii* Clke., having the percentage occurrence of 23.5, and occurring in almost all the parts of the community. It is a low glabrous slender herb, hardly reaching upto 12 cms. Other plants which are present only frequently are as follows :—

|                                      |                |
|--------------------------------------|----------------|
| <i>Asteracantha longifolia</i> Nees. | 12 per cent.   |
| <i>Caesulia axillaris</i> R.         | 23.5 per cent. |
| <i>Cyperus leucocephalus</i> Retz.   | 12 per cent.   |
| <i>Habenaria marginata</i> Coleb.    | 12 per cent.   |
| <i>Heylandia latebrosa</i> DC.       | 6 per cent.    |
| <i>Sopubia delphinifolia</i> Don.    | 10 per cent.   |
| <i>Leucas linifolia</i> Spr.         | 6 per cent.    |
| <i>Utricularia</i> sp.               | 6 per cent.    |

##### 2. *Typha-Asteracantha* community

This community is met with in good marshy places with clayey soil and is composed of *Typha angustata* Bory and *Asteracantha longifolia* Nees; the percentage number of quadrats in which they occur being 90 and 80, respectively. In the 10 quadrats of 1/3 meter square each which were studied from this particular community, the total number of *Typha* plants was found to be 30, while that of *Asteracantha* was 27. But the *Typha* is so prominent, that at first sight,



Fig. 1. Marshy places along the Mutha River at Vitthalwadi.

Fig. 2. Bhosari Lake along Poom-Nasik Road.

Fig. 3. Close-up of *Fimbristylis-Cyperus* community at Vitthalwadi.

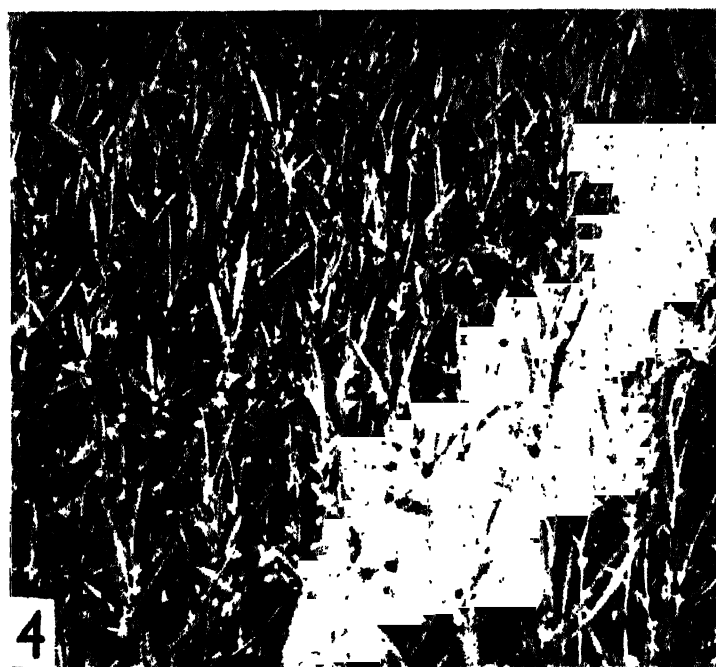


Fig. 4. Pure community of *Caesulia axillaris* along Poona-Sholapur Road.  
Fig. 5. Mixed community of *Caesulia axillaris* at Talegaon.

the community appears to be a pure *Typha* community. It is a robust plant, the height of which is generally between  $1\frac{1}{2}$  and  $3\frac{1}{2}$  meters. Other two species occurring in considerable frequency but with inconspicuous appearance are *Digitaria royleana* and *Cyperus fuscus*, their percentage number of quadrats being 50 and 30, respectively. Such communities are not common, and the authors have come across only one large community along Poona-Nasik road at about the 4th mile from Kirkee. The main reason for this rarity is that *Typha* requires good alluvial soil of considerable depth, and plenty of water.

### 3. *Caesulia axillaris* community

Almost pure communities of *Caesulia axillaris* R. are found in small shallow pools and water-logged areas. It is a succulent erect or suberect herb, 15 to 45 cms. high. The flowers are pale-blue or white and they are borne in sessile, axillary heads. In all 14 quadrats of  $\frac{1}{3}$  meter square were studied and the total number of plants was 95. The percentage number of quadrats in which the species occur is 84. This community is generally restricted to small areas in which stagnant water is available (Fig. 4).

### 4. *Asteracantha*-*Caesulia* community

*Asteracantha longifolia* Nees.—*Caesulia axillaris*, R. community (Fig. 5) is found more frequently than pure communities of each of the two species. The percentage occurrence of the two species is nearly the same i.e., 70. Two other species occurring in this community are *Cyperus leucocephalus* (20 per cent) and *Apluda varia* (10 percent), and they are generally present along the borders of the community. *Asteracantha longifolia* is a stout spiny herb with usually unbranched erect stems 20 cms. to 100 cms. high. It can survive in comparatively drier areas for a longer time; so, after the monsoon is over, it naturally dominates *Caesulia axillaris*. *Asteracantha*—*Caesulia* community is extremely variable, ranging from pure community of these two species to a mixed community in which more than a dozen species occur in a good percentage. At Bhosari, for example, we have studied a community in which both *Caesulia* and *Asteracantha* are 40 per cent, and in addition are the following species with their respective percentage occurrences :—

|                                      |    |
|--------------------------------------|----|
| <i>Acanthospermum hispidum</i> DC.   | 20 |
| <i>Andropogon pumilus</i> Roxb.      | 10 |
| <i>Achyranthes aspera</i> Linn.      | 30 |
| <i>Cymbopogon martini</i> Stapf.     | 10 |
| <i>Cyperus eleusinoides</i> Kunth.   | 20 |
| <i>Cyperus globosus</i> All.         | 10 |
| <i>Cyperus leucocephalus</i> Retz.   | 10 |
| <i>Cyperus nutans</i> Vahl.          | 10 |
| <i>Fimbristylis aestivalis</i> Vahl. | 30 |
| <i>Fimbristylis diphylla</i> Vahl.   | 20 |
| <i>Isachne australis</i> B.Rr.       | 20 |
| <i>Sopubia delphinifolia</i> G.Don.  | 10 |

### 5. *Caesulia axillaris*—*Eleocharis capitata* community

This is a variable mixed community in which a number of other species are frequently present. The percentage occurrence of *Caesulia* and *Eleocharis* is 36.4 and 33 respectively. A good number of species belonging to Cyperaceae are present in this community; and the dominance of Cyperaceae over *Caesulia* is

TABLE I

Chart showing the percentage number of quadrats

| Locality :                                      | Vithalwadi<br>5.2 miles from<br>Poona.                             | Sholapur Road<br>Kunjarwadi<br>14.3 miles from<br>Poona.                       | Sholapur Road<br>Saizpur<br>21.6 miles from<br>Poona.                               |
|---|--|--|---|
| Condition of the soil :                         | Marshy area,<br>shallow sticky<br>brownish soil.                   | Marshy area,<br>deep black soil<br>mixed with sand<br>and gravel.              | Marshy area,<br>shallow sticky<br>block soil, small<br>amount of sand<br>and humus. |
| Other features :                                | Seepage water<br>comes from the<br>canal (Khadak-<br>wasla canal.) | Mostly seepage<br>water from<br>canal. Small<br>amount of drain-<br>age water. | Small pool,<br>marshy place<br>restricted to a<br>small area.                       |
| Number of quadrats<br>studied and their<br>size | 17 quadrats<br>1 meter<br>square each                              | 11 quadrats<br>1/3 meter<br>square each  | 14 quadrats<br>1/3 meter<br>square each   |
| Name of the species:                            |  | Percentage number of quadrats  |   |
| <i>Acanthospermum hispidum</i> DC.              | —  | —  | —   |
| <i>Achyranthes aspera</i> Linn.                 | —  | —  | —   |
| <i>Andropogon pumilus</i> Roxb.                 | —  | —  | —   |
| <i>Apluda varia</i> Hack.                       | —  | 9  | —   |
| <i>Asteracantha longifolia</i> Nees.            | 12   | —  | —   |
| <i>Caesulia axillaris</i> R.                    | 23.5   | 36.4   | 84  |
| <i>Chloris barbata</i> Sw.                      | —  | —  | —   |
| <i>Cyanotis fasciculata</i> Schult.             | 17.7   | —  | —   |
| <i>Cymbopogon martini</i> Stapf.                | —  | —  | —   |
| <i>Cyperus eleusinoides</i> Kunth.              | —  | 18   | —   |
| <i>Cyperus fuscus</i> Linn.                     | —  | —  | —   |
| <i>Cyperus globosus</i> All.                    | 35.4   | 18   | —   |
| <i>Cyperus leucocephalus</i> Retz.              | 12   | —  | —   |
| <i>Cyperus nutans</i> Vahl.                     | —  | 9  | —   |
| <i>Digitaria royleana</i> Prain.                | —  | —  | —   |
| <i>Eleocharis capitata</i> Br.                  | —  | 33   | —   |
| <i>Eragrostis tenella</i> Beauv.                | —  | —  | —   |
| <i>Eriocaulon luzulaefolium</i> Mart.           | —  | 9  | —   |
| <i>Fimbristylis aestivalis</i> Vahl.            | —  | 9  | —   |
| <i>Fimbristylis diphylla</i> Vahl.              | 53   | —  | —   |
| <i>Fimbristylis monostachya</i> Hassk.          | —  | 18   | —   |
| <i>Fimbristylis spathacea</i> Roth.             | —  | 18   | —   |
| <i>Fimbristylis woodrowii</i> Clke.             | 23.5   | —  | —   |
| <i>Habenaria marginata</i> Coleb.               | 12   | —  | —   |
| <i>Heylandia latebrosa</i> DC.                  | 6  | —  | —   |
| <i>Heteropogon contortus</i> Roem.              | —  | —  | —   |
| <i>Isachne australis</i> R.Br.                  | —  | —  | —   |
| <i>Leucas linifolia</i> Spreng.                 | 6  | —  | —   |
| <i>Portulaca</i> sp.                            | 17.7   | —  | —   |
| <i>Sopubia delphinifolia</i> G.Don.             | 10   | —  | —   |
| <i>Typha angustata</i> Bory.                    | —  | —  | —   |
| <i>Utricularia</i> sp.                          | 6  | —  | —   |

TABLE I (contd.)

*in which the species occur in the various localities*

| <i>Bhosari</i><br>5 miles from<br>Kirkoo on<br>Poona-Nasik<br>Road. | <i>Poona-Nasik Road</i><br>4.2 miles from<br>Kirkee. | <i>Talegaon</i><br>Near Ghorwadi<br>Station.    | <i>Talegaon</i><br>Near the lake.                                   | <i>Poona-Nasik Road</i><br>3 miles from<br>Poona. |
|---|--|---|---|---|
| Marshy area,<br>deep sticky<br>black soil.                          | Marshy area,<br>deep sticky<br>brownish soil.        | Marshy area,<br>deep brownish<br>gravelly soil. | Marshy area,<br>shallow sticky<br>and sandy soil.                   | Marshy area,<br>brownish gravelly<br>soil.        |
| Marsh along a<br>lake. Large<br>area.                               | Marshy area<br>along a water<br>stream.              | Small pools.                                    | A large lake.<br>Small patches of<br>marshy area<br>along the lake. | Small patches<br>near shallow<br>pools.           |
| 10 quadrats<br>1/3 meter<br>square each                             | 10 quadrats<br>1/3 meter<br>square each              | 10 quadrats<br>1/3 meter<br>square each         | 10 quadrats<br>1/3 meter<br>square each                             | 10 quadrats<br>1/3 meter<br>square each           |
| <i>in which the species occur :—</i>                                |  |   |   |   |
| 20  | —  | —   | —   | —   |
| 30  | —  | —   | —   | —   |
| 10  | —  | —   | —   | 90  |
| —   | —  | 10  | 10  | —   |
| 40  | 80   | 70  | 20  | 20  |
| 40  | —  | 70  | 30  | 60  |
| —   | —  | —   | —   | —   |
| 10  | —  | —   | —   | —   |
| 20  | —  | —   | —   | —   |
| 10  | 30   | —   | —   | —   |
| 10  | —  | —   | —   | 20  |
| 10  | —  | 20  | —   | —   |
| 10  | —  | —   | —   | —   |
| 20  | 50   | —   | 20  | —   |
| —   | —  | —   | —   | —   |
| —   | —  | —   | 20  | —   |
| —   | —  | —   | —   | —   |
| 30  | —  | —   | —   | 30  |
| 20  | —  | —   | —   | —   |
| —   | —  | —   | —   | 20  |
| —   | —  | —   | —   | —   |
| —   | —  | —   | —   | —   |
| —   | —  | —   | —   | —   |
| —   | —  | —   | —   | —   |
| —   | —  | —   | —   | 60  |
| 20  | —  | —   | —   | —   |
| —   | —  | —   | —   | —   |
| —   | —  | —   | —   | —   |
| 10  | —  | —   | 10  | 20  |
| —   | 90   | —   | —   | —   |
| —   | —  | —   | —   | —   |



well-marked. The following other species were observed in marshy area at Sholapur Road :—

|   |              |
|---|--------------|
| 1. <i>Cyperus globosus</i> All.           | 18 per cent. |
| 2. <i>Fimbristylis monostachya</i> Hassk. | 18 per cent. |
| 3. <i>Fimbristylis aestivalis</i> Vahl.   | 9 per cent.  |
| 4. <i>Fimbristylis spathacea</i> Roth.    | 18 per cent. |
| 5. <i>Cyperus nutans</i> Vahl.            | 9 per cent.  |
| 6. <i>Cyperus eleusinoides</i> Kunth.     | 18 per cent. |

Besides these, grass namely *Apluda varia* and *Eriocaulon luzulaefolium* occur in small percentage.

#### 6. *Andropogon*—*Caesulia*—*Heteropogon* community

The locality in which this community was studied is not strictly marshy or swampy. In some places near pools, it may attain marshy conditions ; but the major part is just a moist grassland.

*Andropogon pumilus* is the most abundant and dominant feature in the vegetation, being present in 90 per cent of the total quadrats studied.

*Caesulia axillaris* and *Heteropogon contortus* both occur in the community in 60 per cent of the quadrats. *Caesulia axillaris* is more abundant in the wetter parts, while *Heteropogon* is prominent in the drier situations. The other species occurring in this community are given below with their respective percentages :—

|  |    |
|--|----|
| <i>Asteracantha longifolia</i> Nees.   | 20 |
| <i>Cyperus globosus</i> All.           | 20 |
| <i>Fimbristylis aestivalis</i> Vahl.   | 30 |
| <i>Fimbristylis monostachya</i> Hassk. | 20 |
| <i>Sopubia delphinifolia</i> G. Don.   | 20 |

#### 7. *Ipomea aquatica* Forsk. community

In the shallow water and moist mud along the border of Bhosari lake (Poona-Nasik Road) extensive patches of *Ipomea* community are present. The plant is a runner forming a dense network of branches. It grows only in shallow water and where enough amount of soil is present.

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DEVELOPMENT OF THE SKULL IN CATFISHES  
PART V. DEVELOPMENT OF SKULL IN *HETEROPNEUSTES FOSSILIS*  
(BLOCH)

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ABSTRACT

Five stages in the development of the skull and the adult skull have been described by using modern nomenclature as adopted by de Boer (1937).

The origin of some of the disputed bones of the siluroid skull has been traced and discussed in the paper. The maxillae and premaxillae are seen to develop fairly early during the development i.e., 8 mm. and 12 mm. larvae respectively. The ethmoid bone which arises as a perichondral ossification of the lamina procerebralis of the chondrocranium possesses membranous extensions but no separate membrano ossifications co-ossifying with it. The bone develops in the 29 mm. stage. The nasals which develop in the 33 mm. stage arise as membrane bones surrounding the anterior ends of the supraorbital sensory canals.

In the 19 mm. stage the parasphenoid and the frontals develop as membrane bones and the prootic, supraoccipital, basi-occipital and quadrato develop as perichondral ossifications. The supraoccipital bone which arises as a single centre of ossification from the tectum synoticum extends laterally and anteriorly. A parietal ossification is never noticed and the supraoccipital ossification extends to the region of the parietals.

The angular and dentary bones arise in the 19 mm. stage around Meckel's cartilage and no part of Meckel's cartilage is invaded by any ossification in any stage of development. Splenial bone is absent. In the 21 mm. stage the exoccipital, lateral ethmoids, sphenotics and pterotics with the supratemporal extension develop as perichondral ossifications. A posttemporal around the sensory canal is also seen to develop in this stage. The lateral ethmoids have ventral extensions which are connected with the orbitosphenoids. In the 33 mm. stage the plourosphenoids, orbitosphenoids, hyomandibulae, metapterygoids and palatines are developed as perichondral ossifications. The other bones that develop in membrane in this stage are the prevomer, supraorbitals, nasals, oporeles, preoporeles and interoporeles. The orbitosphenoids are seen to develop as paired perichondral ossifications in the 33 mm. stage and in the 49 mm. stage the two ossifications fuse to form a median bone. The intertemporal bones and suborbitals are seen to develop last in the series of the bones. The parietals, opisthotics, opiotics, symplectics, suboporeles and basisphenoid bones are absent.

The lateral line system and the hyobranchial skeleton are described. In the branchial arches there are the second and third basibranchs. The pharyngobranchs of third and fourth arches are separate. The opibranchs of all the four arches are distinct. The hypobranchs of third and fourth arches are represented by cartilage. The urohyal (parahyoid) is a large bone having a pair of long pointed lateral processes.

INTRODUCTION

In an earlier paper (Srinivasachar, 1957b) the development of chondrocranium has been completely described in *Heteropneustes* and in this paper an attempt has been made to describe the development of the osteocranium with a view to find out the origin of some of the disputed bones of the siluroid skull. Kindred (1919) has given a detailed account of the various bones of skull in *Amiurus*. Earlier McMurrich (1884) and Herrick (1901) have described the topographical relations of the cranial bones of the adult *Amiurus*. More recently Bhimachar (1933) has given an account of the morphology of the skull of certain Indian siluroids. David (1935, 1936) has described the skull of the African members of the siluroid families Calariidae and Bagridae and also has figured the dorsal aspect of the skull of

*Saccobranchus fossilis*. In all these cases it appears that the nomenclature of the bones is not in conformity with the development of the different bones. I have used in my description of the skull as far as possible the nomenclature adopted by de Beer (1937).

#### MATERIAL AND METHODS

The material presented in this paper consists of a number of developing stages and adults of *Heteropneustes* collected round about Bangalore and Trivandrum. Some of the early stages were reared in the laboratory. Alizarin transparencies were made for both the developing larvae and adults in order to study the centres of ossifications and also the topography of various bones of the skull. In certain cases slightly higher percentage of KOH was used in order to disarticulate the bones to study the individual bones in relation to the neighbouring ones. Differential staining of bones and cartilages was also used in some early stages to study the cartilage and membrane bones. The following stages have been studied in the development of skull in *Heteropneustes*.

| Stage    | Total-length | Head-length | Remarks                |
|----------|--------------|-------------|------------------------|
| 1.       | 19.0 mm.     | 3.5 mm.     | Differential staining. |
| 2.       | 21.0 mm.     | 4.0 mm.     | Alizarin.              |
| 3.       | 29.0 mm.     | 5.0 mm.     | Alizarin.              |
| 4.       | 33.0 mm.     | 5.5 mm.     | Alizarin.              |
| 5.       | 49.0 mm.     | 9.0 mm.     | Alizarin.              |
| 6. Adult | 210.0 mm.    | 33.0 mm.    | Alizarin.              |

Adult skull of *Clarias batrachus* was also studied for the sake of comparison.

#### OBSERVATIONS

The adult skull of *Heteropneustes* is well ossified and compact, showing dorso-ventral compression. It also shows pitted appearance on the dorsal surface. The sutures separating the various bones are very clear and prominent. In the adult skull very little cartilage remains unossified and practically the cranium is free from cartilage. The large fontanelle noticed in the chondrocranium (Srinivasachar, 1957b) is almost completely closed except for a small anterior one between the frontals and a posterior one in the supraoccipital bone.

I shall now describe the skull in the following order.

##### A. The cranium

1. Ethmoid region.
2. Orbitotemporal region.
3. Auditory region.
4. Occipital region.

##### B. The jaws and hyobranchial skeleton

1. Upper jaw.
2. Lower jaw.
3. Hyoid arch.
4. Branchial arches.

### A. The cranium.

1. *The ethmoid region* : The ethmoid region in the adult skull is formed by a median ethmoid bone and paired lateral ethmoids with dorsal nasals and ventral prevomer bones.

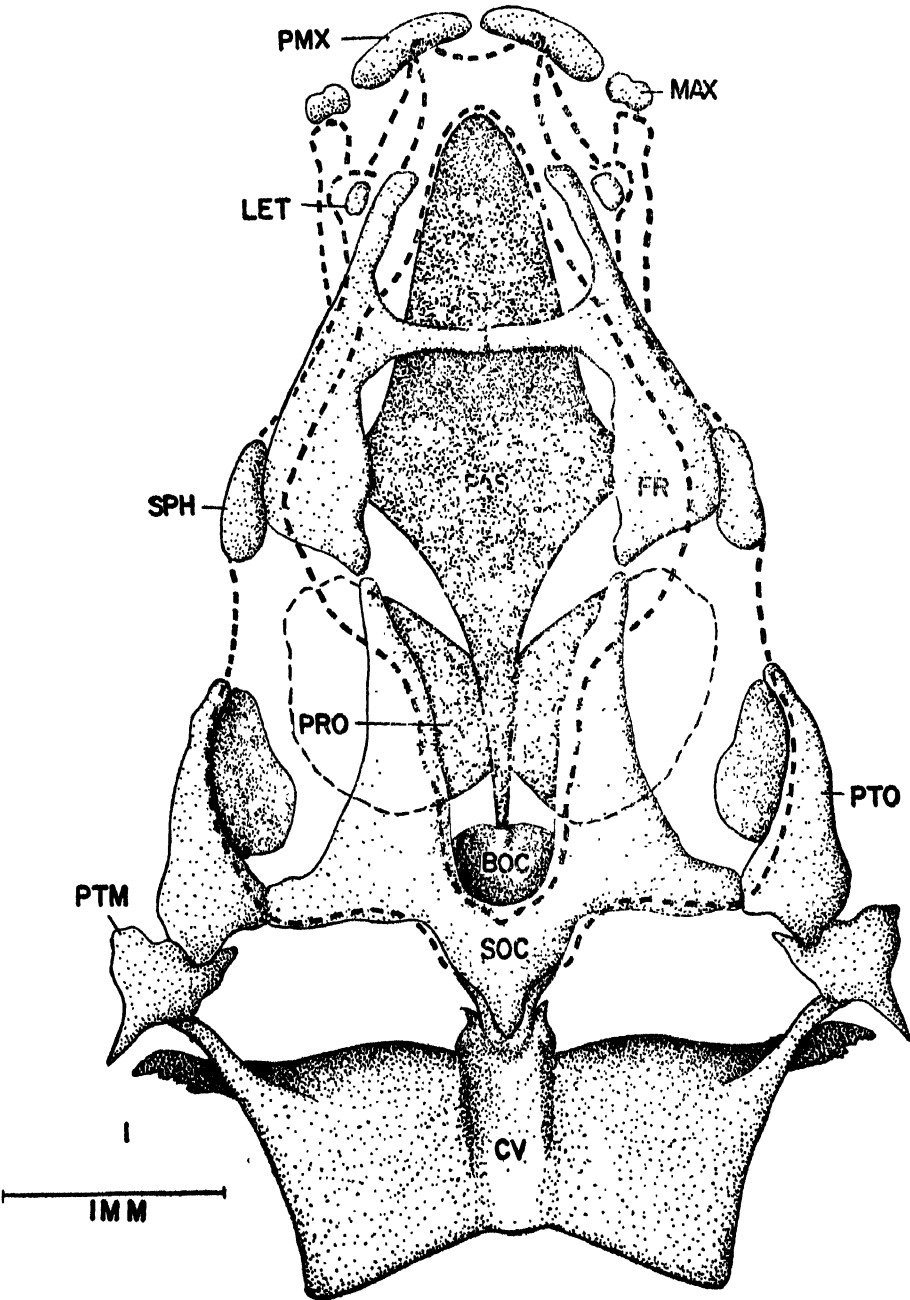
*The ethmoid bone* (Text-figs. 3, 4, 6, 7 and 9a, ETH) : The ethmoid bone forms the anterior terminal bone of the cranium. It is flat dorsally and anteriorly shows a pair of processes which can be recognised as the pre-ethmoid cornua. These cornua are quite evident in the chondrocranium (Srinivasachar, 1957b). The ethmoid ossification is seen as perichondral lamella of the lamina precerebralis in a chondrocranium of the 29 mm. stage and in the 33 mm. stage, the bone is almost completely ossified. I have not been able to see any separate membranous ossification associated with the perichondral ossification. Hence, I prefer to designate the bone as merely an ethmoid bone. This bone has been variously called by different authors without regard to the significance of the terminology used. Kindred (1919) described the bone as supraethmoid as done by Allis (1910) in *Loricati*. Anteriorly the ethmoid cornua extend over the premaxillae with which they are not connected. The ethmoid is connected by means of sutures laterally and ventrally with the lateral ethmoids and posteriorly with the frontals and it forms almost the anterior boundary of the anterior fontanelle.

*The lateral ethmoids* (Text-figs. 1, 3, 4, 6, 7 and 9a, LET) : The bone is developed in the region of the lamina orbitonasalis and the ossification is seen in the 21 mm. larva in the form of a small oval piece on the dorsal side of the lamina orbitonasalis. The bone therefore arises first as a perichondral ossification and in the adult skull each lateral ethmoid is a large bone having a prominent ventral process. It forms the posterior boundary for the nasal fossa. Dorsally the lateral ethmoid articulates with the ethmoid, mesially with the frontal and posteriorly with the supraorbital and frontal bones. The ventral process of the lateral ethmoid is connected by a piece of cartilage with the orbitosphenoid posteriorly and anteriorly with the ethmoid. Kindred (1919) described this bone as the ectethmoid in *Amiurus*. Both the olfactory nerve and superficialis branches of the trigeminal nerve pass through foramina in the lateral ethmoid ventrally.

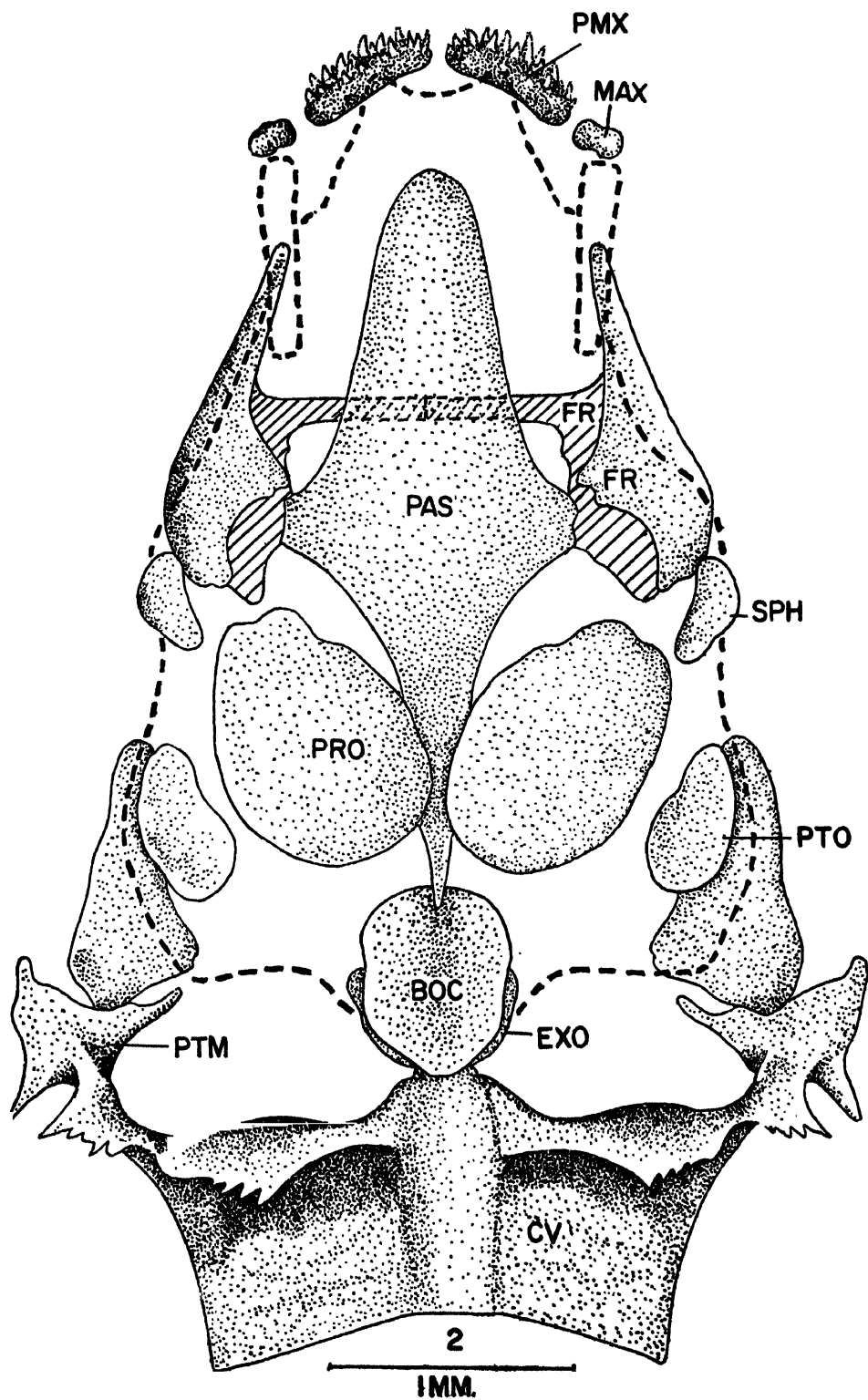
*The nasals* (Text-figs. 3, 4, and 6, NS) : These bones are developed on the dorsal side of the nasal fossae around the anterior end of the supraorbital sensory canal in the 33 mm. stage. Each nasal bone is in the form of a tube having anterior and posterior openings and forms a sort of incomplete roof for the nasal capsule. Dorsally the nasal bone is almost isolated from other bones but the sensory canal passes from the posterior tip of the bone through a piece of connective tissue into the frontal. The nasals of *Heteropneustes* resemble to a large extent those of Cyprinoids (Ramaswami, 1955). But nasals of the Characiniidae (Sagemehl, 1885) are more like those in *Amia* (Sagemehl, 1884) where the bone comes in contact with the ethmoid cartilage. In *Clarias* (George, 1954) the nasal is a flat bone fitting laterally into a groove in the ethmoid bone.

*The prevomer* (Text-figs. 4, 7 and 9a, PO) : The prevomer arises as an unpaired bone on the ventral surface of the anterior region of the skull. The bone is observed to develop in the 33 mm. stage behind the ventral extension of the ethmoid bone and in this stage the prevomerine teeth are still lacking. The median process of the prevomer in the above stage is very short. The teeth appear to develop in later stages independently of the bone. In the adult skull the prevomer is in the form of a T-shaped bone having a pair of prominent patches of teeth on the horizontal portion of the bone. The teeth are all of uniform shape and size. The vertical portion of the bone tapers posteriorly and fits into the anterior forked portion of the parasphenoid bone. Anteriorly the prevomer does not articulate with the premaxillaries.

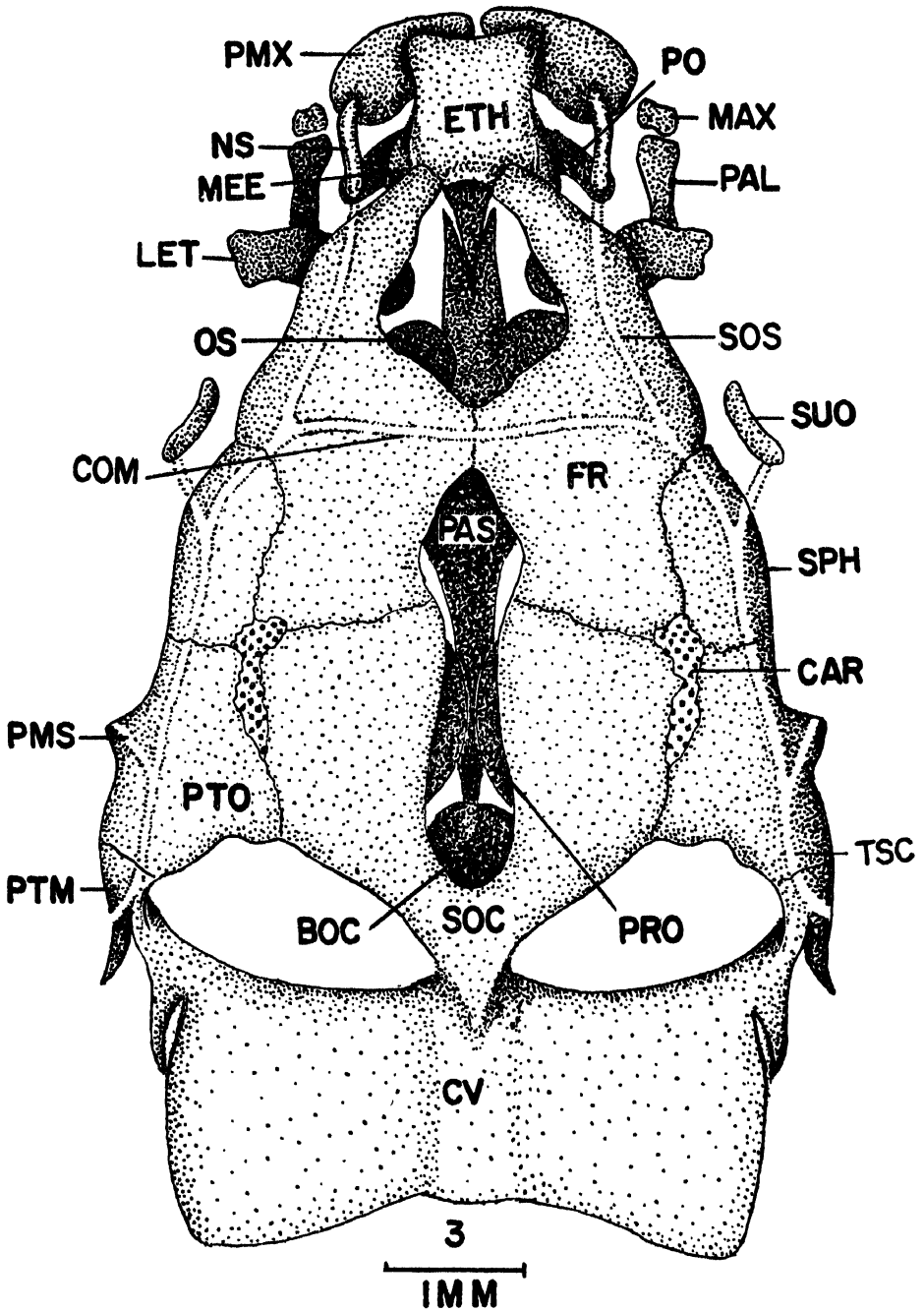
2. *The orbitotemporal region* : The region behind the orbit can be recognised as the sphenoid region. On the dorsal side there are a pair each of large frontals



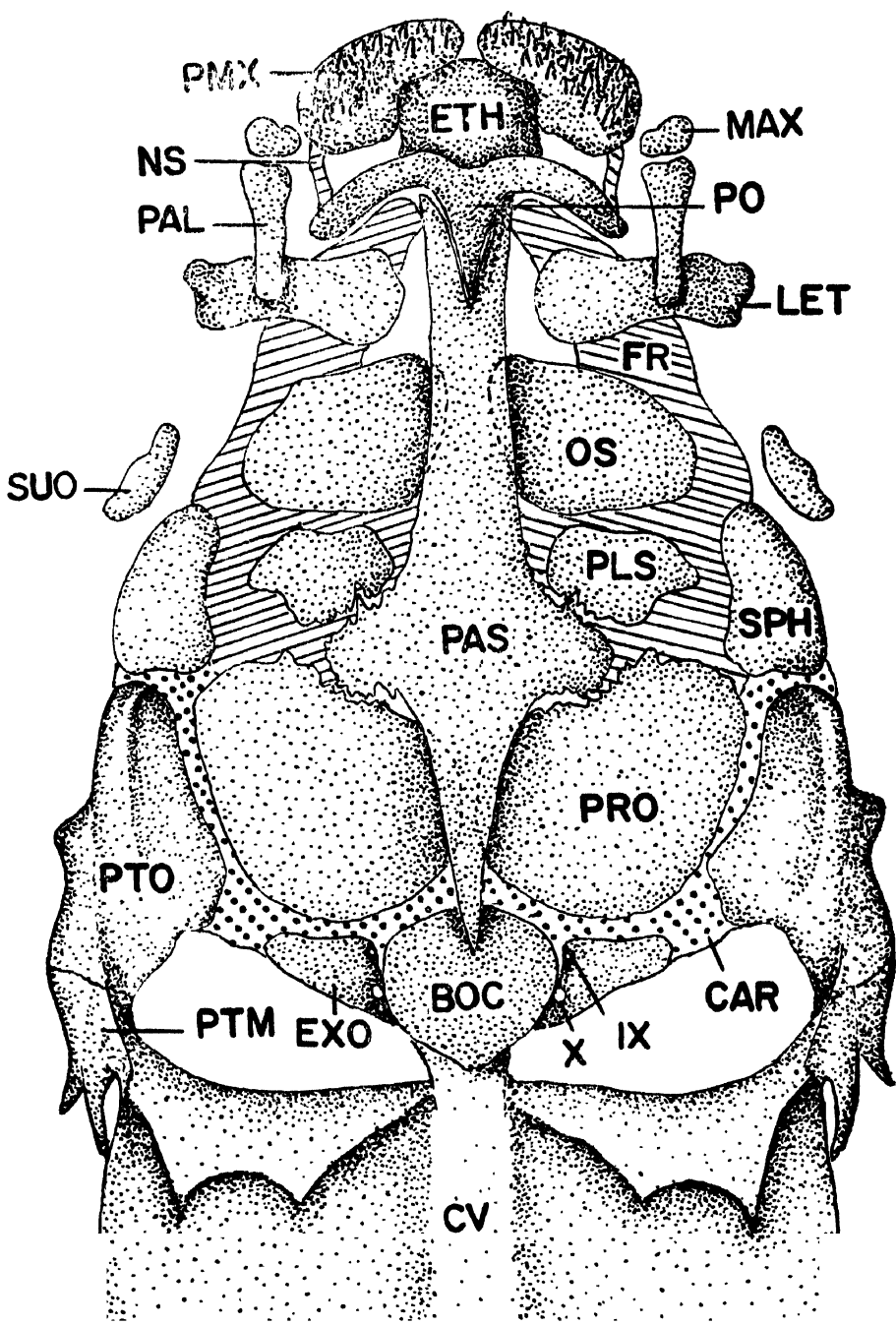
Text-fig. 1. Dorsal view of the skull of 21 mm. larva of *Heteropneustes fossilis* (Bloch). The outline of the chondrocranium has been shown by thick broken line.



Text-fig. 2. Ventral view of the same.



Text-fig. 3. Dorsal view of the skull of 33 mm. larva of *Heteropneustes fossilis* (Bloch).



**IMM**

Text-fig. 4. Ventral view of the same.



and supraorbitals with the sub-orbital series of bones and ventrally are present the parasphenoid, orbitosphenoids and pleurosphenoids.

*The frontals* (Text-figs. 1, 2, 3, 4, 6, 7 and 9a, FR): The frontals are large bones arranged on either side of the mid-dorsal line of the skull. The frontal ossification is first seen in the 19 mm. larva forming a roof over the large fontanelle of the chondrocranium. The frontal ossifications in the 19 mm. and 21 mm. stages extend mesially in the form of tubular extension around the sensory canal at about the middle region of the chondrocranium and the ossifications are separated by a thin suture. Anteriorly the ossification extends to the level of the lamina orbitonasalis of the chondrocranium around the anterior extension of the supraorbital sensory canal. These ossifications are posteriorly separated from the ossification of the supraoccipital bone. In the 33 mm. stage the frontal ossification extends both anteriorly and posteriorly and the positions around the commissural sensory canal have also broadened thus dividing the dorsal fontanelle into an anterior broad and a posterior narrow fontanelle. The two frontal ossifications do not meet anteriorly in front of the anterior fontanelle at this stage. In the adult skulls the frontals are large flat bones with a small anterior fontanelle between them. Anteriorly they are connected suturally with the median and lateral ethmoids, laterally with the supraorbital and sphenotic bones and posteriorly with the pterotic and supraoccipital bones. Each frontal bone extends mesioventrally and is connected by sutures with the orbitosphenoid and pleurosphenoid of its side.

*The parasphenoid* (Text-figs. 1, 2, 3, 4, 7 and 9a, PAS): This bone is a long flat bone extending on the ventral surface of the cranium. In the 19 and 21 mm. stages the parasphenoid ossification is seen ventral to the hypophysial fenestra of the chondrocranium. Anteriorly the ossification is round in shape and extends as far as the posterior end of the lamina precerebralis. In the middle region, the ossification extends as lateral processes and posteriorly tapers between the two prootic ossifications. In the 33 mm. larva the anterior end of the parasphenoid ossification shows a forked appearance and into this is fitted the posterior end of the prevomer. In the adult skull, the parasphenoid forms an almost ventral floor for the brain case and extends from the ethmoid to the posterior basioccipital bone. In the region of the optic foramen, the parasphenoid overlies the median portion of the orbitosphenoid bone. The middle portion of the parasphenoid possesses a pair of lateral pointed extensions which form the anterior boundary for the trigeminofacial foramen. The posterior end of the bone extends into the forked portion of the basioccipital bone.

*The orbitosphenoid* (Text-figs. 3, 4, 7 and 9a, OS): It is an unpaired bone in the adult skull. However, it arises in the 33 mm. stage as paired perichondral ossifications in the preoptic roots of the orbital cartilages on either side of the anterior ends of the parasphenoid ossification. These paired ossifications are noticed behind the lateral ethmoids and in front of the optic foramen (Figs. 7, 9a, II). As development advances the perichondral ossification extends in the membrane across the anterior part of the hypophysial fenestra and fuses with the fellow of the opposite side to form a median bone dorsal to the parasphenoid bone. In the adult skull, the orbitosphenoid is a large bone attached ventrally by its median portion with the parasphenoid bone. The bone has laterally a pair of large processes which forms the side wall of the anterior part of the cranial cavity. Anteriorly these lateral portions of the orbitosphenoid are attached to the ventral extension of the lateral ethmoid, dorsally with the frontals and posteriorly with the pleurosphenoid bones.

A basisphenoid (suprasphenoid) bone is completely absent.

*The pleurosphenoids* (Text-figs. 4, 7 and 9a, PLS): These are paired bones in the adult skull and in the 33 mm. stage the bones arise partly as perichondral ossification in the orbital cartilage and partly in the membrane of the side wall of the cranial cavity in front of the exit of the trigeminal nerve. Posteriorly these

ossifications are suturally connected with the lateral extensions of the parasphenoid bone. In the adult skull the pleurosphenoids are flat bones slightly bulged in the middle extending between the optic and trigeminal foramina. These bones form the side wall for the cranial cavity posterior to the orbitosphenoids. They are connected anteriorly with the orbitosphenoids, posteriorly with the lateral extensions of the parasphenoid and laterally with the sphenotic bones.

A myodome for insertion of the recti muscles is absent in *Heteropneustes* as in other siluroids.

*The supraorbitals* (Text-figs. 3, 4, 6 and 7, SUO): The bone arises for the first time in the 33 mm. stage in a membrane behind the orbit and anterior to the sphenotic ossification, surrounding the sensory canal branch given off from the supraorbital sensory canal. In the adult skull the supraorbital bone forms the dorsal boundary for the orbit and is connected by sutures anteriorly with the lateral ethmoid, laterally with the frontal and posteriorly with the sphenotic and intertemporal bones.

*The suborbitals* (Text-figs. 6, 7, AOR, LAC, SO1, SO2): This series consists of a chain of four bones forming the lower boundary of the orbit. None of them has appeared in the developing stages described. They appear to develop late in ontogeny around the suborbital sensory canal which continues from the supraorbital bone. The anterior of the four bones is a small oval piece lying in the connective tissue in the posterior part of the maxillary bone and could be designated as the antorbital (AOR) bone. Posterior to this antorbital bone is another one which is probably a lacrimal (LAC) in which the sensory canal ends, and the other two bones of the chain are large dorso-ventrally compressed ones and may be called the suborbitals (SO1 and SO2).

3. *The auditory region*: In the adult skull this region is formed by the prootics, sphenotics, pterotics and associated with these are the intertemporal bones. The latter bones are completely excluded from the auditory capsules.

*The prootics*: (Text-figs. 1, 2, 3, 4, 6, 7 and 9a, PRO): These bones are fairly large ones forming the floor and the lateral wall of the cranial cavity behind the pleurosphenoids and on either side of the median parasphenoid. The prootic bones arise as flat perichondral ossifications in the 19 mm. and 21 mm. stages in the anterior part of the parachordals and ventral portion of the otic capsules behind the hypophysial fenestra. Gradually in the 33 mm. larva the prootic ossifications extend inwards to meet the fellow of the opposite side dorsally to the posterior part of the parasphenoid bone. In the adult skull each prootic extends from the trigeminal (Text-figs. 7, 9a, V) to the glossopharyngeal foramina (IX). The bone has an irregular outline and is not pierced by any nerve or blood vessel. Anteriorly the prootic bone is connected by dovetails with the parasphenoid, laterally it is firmly connected with ventral extensions of the sphenotic and pterotic, and posteromesially with the exoccipital bones. Externally the bone is smooth and internally accommodates the otolith (Text-fig. 7, OT) which is visible through the bone.

*The sphenotics* (Text-figs. 3, 4, 6, 7 and 9a, SPH): These are situated in the postero-lateral region of the frontal bones. They appear comparatively to be small bones. In the 21 mm. stage the bones are seen as perichondral ossification in the region of the anterior semicircular canal of the auditory capsule. In the 33 mm. stage the ossification extends to the frontal anteriorly, to the pterotic posteriorly and to the prootics ventrally. In the adult skull the sphenotic shows a ventral extension forming a sort of anterior boundary for the auditory capsule. The facial foramen (Text-figs. 7, 9a, VII) is noticed between the ventral extension of the sphenotic and prootic bones.

*The pterotics* (Text-figs. 3, 4, 6, 7 and 9a, PTO): These bones are situated on either side of the median supraoccipital and are posterior to the sphenotic bones. In the 21 mm. larva the pterotics appear as perichondral ossifications in the region

of the posterior semicircular canal and dorsal to each perichondral ossification is seen a membranous ossification around the sensory canal, probably of the supratemporal co-ossifying with it. I have not been able to see an independent ossification of the supratemporal but it appears to develop along with the perichondral ossification. I have therefore not given a compound name to the pterotic bone. Further development of the bone is seen in the 33 mm. stage when dorsally the pterotic bone meets anteriorly the sphenotic and posteriorly the post-temporal. In the adult skull the pterotic bone is an extensive bone surrounded by the sphenotic, intertemporal, post-temporal and supraoccipital bone. Ventrally the pterotic has an extension forming the posterior and lateral portion of the auditory capsule probably taking the place of the opisthotic bone.

The epiotic and opisthotic bones are absent in *Heteropneustes*.

*The intertemporals* (Text-figs. 6 and 7, INT) : These have been named by a number of previous authors including Gregory (1933) and George (1954), as the dermal representative of the sphenotic bone. The intertemporal is not developed in any stages earlier than the adult skull. In the adult skull the bone is completely excluded from the otic capsule and is found between the supraorbital and the posttemporal bones. The intertemporal appears to develop outside the auditory capsule in the membrane around the sensory canal branch given off from the supraorbital sensory canal (Text-figs. 3, 6, SOS) in the pterotic bone. It is attached suturally with the postorbital anteriorly, posteriorly with post-temporal and laterally with sphenotic and pterotic bones.

4. *The occipital region* : This region of the adult skull is formed as in other teleosts by four bones ; dorsally by a large supraoccipital, ventrally by the basioccipital and laterally by a pair of exoccipital bones around the foramen magnum.

*The supraoccipital* (Text-figs. 1, 3, 6, and 9a, SOC) : The bone is a fairly large dorso-medial bone forming the posterior end of the adult skull. In the 19 mm. and 21 mm. stages the supraoccipital ossification is seen in the region of the tecti synoticum and posterior of the chondrocranium and extends anteriorly on either side of the postero-lateral border of the dorsal fontanelle. The ossification extends anteriorly to meet the frontals, laterally to meet the supratemporal-pterotic ossifications and posteriorly extends as a supraoccipital spine. In the 33 mm. stage the ossification has further developed anteriorly and have joined the frontals on either side of a narrow posterior fontanelle, but separated from the pterotic by a strip of cartilage. The entire supraoccipital bone is formed by a single centre of ossification and a separate parietal ossification is not noticed in any of these stages. The supraoccipital has extended into the place of the parietal bone. In the adult skull the supraoccipital retains a very small posterior portion of the dorsal fontanelle in its posterior end. The bone has a long pointed spine (Text-figs. 6, 7 and 9a, SPO) almost equal in length of the entire bone. This spine extends on the dorsal surface of the united vertebrae (complex vertebrae).

*The basioccipital* (Text-figs. 1, 2, 3, 6, 7 and 9a, BOC) : This bone forms the posterior end of the cranium on the ventral surface. It arises in the 19 mm. and 21 mm. stages as an ossification in the posterior part of the parachordal forming the floor for the saccular recesses. In these stages and also in the 33 mm. stage the bone is almost of a triangular shape and the anterior portion overlaps the posterior pointed extension of the parasphenoid bone. Gradually in the adult skull the bone becomes extensive and possesses anteriorly a deep wedge into which fits the posterior end of the parasphenoid. Antero-laterally, the basioccipital is connected suturally with the prootics and postero-laterally with the exoccipitals. Internally the bone possesses a cavity for the accommodation of the utriculus (Text-fig. 9a, OTU) but externally the bone is smooth. The posterior end of the bone articulates with the anterior end of the centrum of the complex vertebrae (Text-figs. 1, 2, 3, 6, 7 and 9a, CV).

*The post-temporals* (Text-figs. 1, 2, 3, 4, 6 and 7, PTM) : These bones primarily belong to the pectoral girdle. The bone arises in the 21 mm. stage as an ossification in the region outside the postero-lateral end of the auditory capsule of the chondrocranium and at this stage it is connected with the posterior end of the supratemporal-pterotic ossification. In the 33 mm. stage the ossification extends anteriorly and completely becomes connected with the posterior end of the pterotic bone. This ossification surrounds the portion of the anterior end of the lateral line sensory canal entering the skull. In the adult skull the post-temporal bones are firmly attached to the postero-lateral ends of the skull and possess posteriorly processes. Ventrally the post-temporal bone is connected with the antero-lateral end of the bony capsule (complex vertebrae). Anteriorly the bone is suturally connected with the intertemporal and pterotic bones.

*Lateral line sensory canal* : The lateral line sensory canal entering the skull passes through the post-temporal bone and extends anteriorly in the supratemporal-pterotic bone as the temporal sensory canal (Text-figs. 3, 6, TSC). This canal gives off the preopercle-mandibular sensory canal branch (Text-figs. 3, 5, 6, PMS) in the supratemporal-pterotic bone which passes through the inter-temporal into the preopercle bone. The temporal sensory canal continues anteriorly as the supraorbital canal (SOS) in the sphenotic bone and gives off the infraorbital sensory canal (Text-figs. 6, SC) which extends into the suborbitals. Further the supra-orbital sensory canal passes forwards in the frontal and extends anteriorly into the nasal. The two supraorbital canals are connected behind the anterior fontanelle by means of a commissural sensory canal (COM). The suborbital branch passes through the suborbitals and in the anterior bone of the chain, the lacrimal.

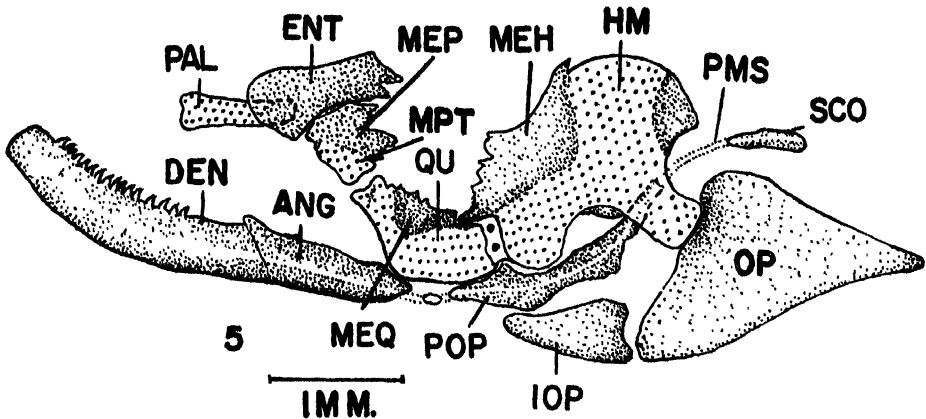
#### B. *The jaws and the hyobranchial skeleton.*

1. *The upper jaw* : In the osteocranium the independent part of the palatoquadratebar or the pterygoid process of the chondrocranium is ossified completely without any membranous extension, into a palatine bone (Text-figs. 3, 4, 5 and 7, PAL). The palatine is in the form of a cylindrical rod slightly swollen at the anterior end and articulates anteriorly with the maxilla. The other ossifications of the palatoquadrate can be clearly made out in the 33 mm. stage. The quadrate ossification (Text-figs. 5, 8, QU) can be seen in the quadrate portion of the hyomandibula of the chondrocranium and associated with it, is a membranous extension dorsally (Text-fig. 5, MEQ) and ventrally it articulates with the lower jaw. A piece of the original cartilage (Text-fig. 8, CAR) between the ossified hyomandibula (HM) and the ossified quadrate is left unossified and persists even in the adult skull. Anterior to the quadrate ossification, is the metapterygoid (MPT) which is also partly perichondral and partly membranous (Text-fig. 5, MEP) and closely attached to the anterior end is another small plate-like bone which is a completely membranous ossification. The latter bone probably represents the entopterygoid bone (ENT). The palatine bone is completely free from the entopterygoid articulation indicating its original independent chondrification of the chondrocranium.

An ectopterygoid bone seen in other teleosts is absent in *Heteropneustes*.

The premaxillae and maxillae which are in the anterior end of the upper jaw are membrane bones. *The premaxillaries* : (Text-figs. 1, 2, 3, 4, 6, 7 and 9a, PMX). These form the anterior end of the upper jaw on each side. Each premaxilla arises in front of the lamina precerebralis of the chondrocranium fairly early in the development and in the 21 mm. larva the bone is already developed in the form of a plate articulating ventrally with the ethmoid cornu of the lamina precerebralis. The ventral surface of the bone is covered by teeth which are pointed and of uniform size. In the 33 mm. stage the premaxillaries show thin posterior extensions which are at this stage poorly ossified. The bones in the adult skull are quite large ones with prominent posterior processes which form a sort of osseous floor for the nasal

sacs. Ventrally they are connected with the osseous ethmoid cornua of the ethmoid bone. Mesially the bones are connected by connective tissue and laterally they are connected with the maxilla.



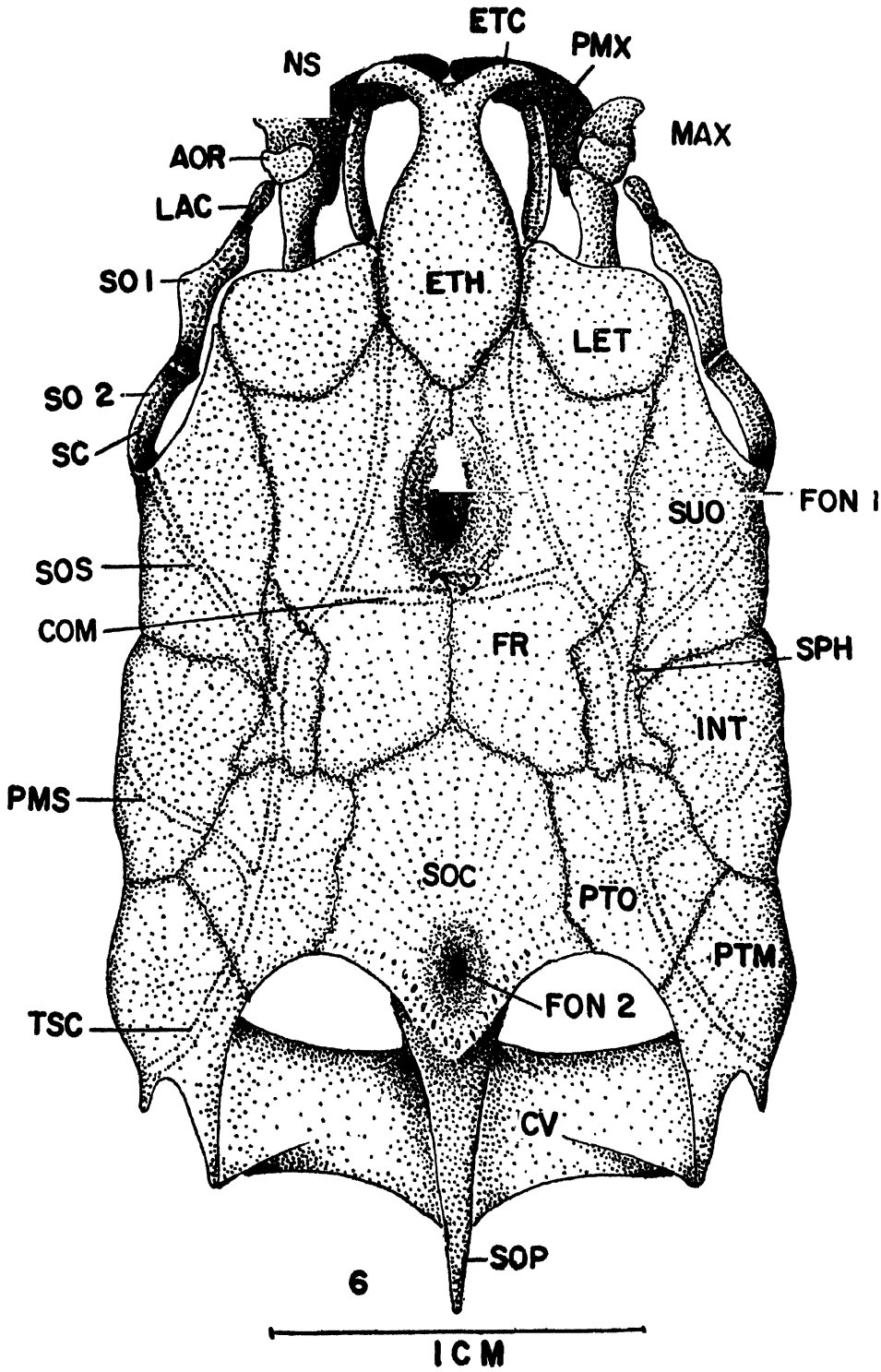
Text-fig. 5. Outer view of the left side of the upper and lower jaws with opercular bones of 33 mm. larva of *Heteropneustes fossilis* (Bloch). The perichondral ossifications are shown as thicker stippled areas.

*The maxillaries* (Text-figs. 1, 2, 3, 4, 6, 7 and 9a, MAX) : The maxillary bone is one of the earliest to develop in the osteocranium. In the 8 mm. larva the maxilla arises as a tiny ossification in front of the pterygoid process of the chondrocranium. In the 21 mm. larva the maxilla is already well formed postero-laterally to the premaxilla on each side. In the 33 mm. stage, when the palatine bone is formed the maxilla articulates with it by means of a hollow depression in the anterior end of the palatine. In the adult skull each maxilla is a small toothless bone which is slightly irregular in shape and supports the maxillary barbel anteriorly. These maxillaries form lateral margins of the upper jaw.

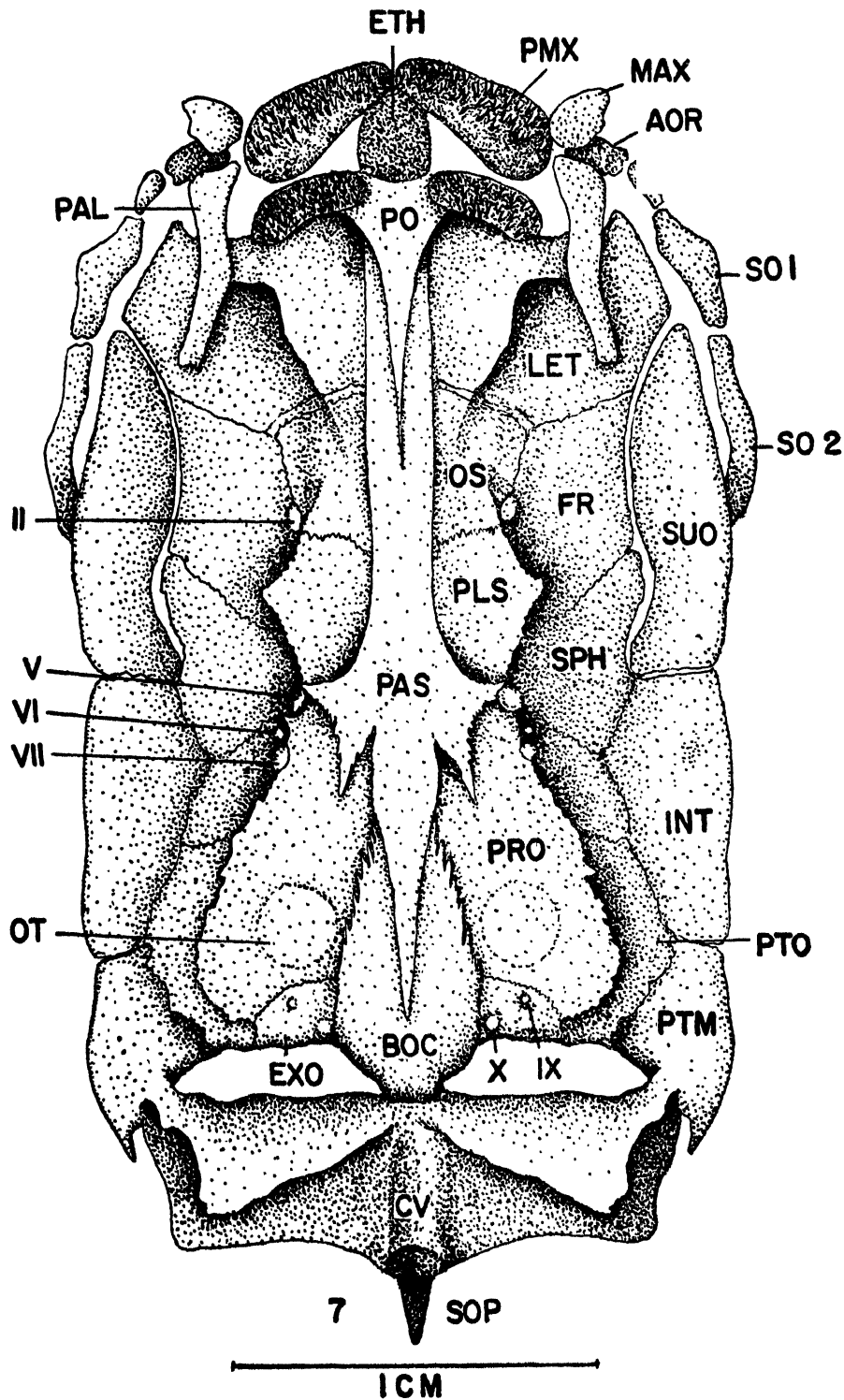
*The lower jaw* : The lower jaw in the adult skull consists of two halves connected mesially by a strip of connective tissue. Each half of the lower jaw consists of two bones, an anterior dentary (Text-figs. 5, 8, DEN) and a posterior angular (ANG) (articular of other authors) which later articulates with the quadrate. Both dentary and angular develop as completely membrane bones around the original Meckel's cartilage of the chondrocranium. No part of the cartilage is invaded by ossification during the development.

The dentary is fairly stout bone, anterior broad and flat and dorso-medially it possesses a number of villiform teeth. The angular bone is more or less of the same size as the dentary and has a socket posteriorly for articulation with the quadrate. Mesially the bone shows a depression in which the persisting Meckel's cartilage could be made out. A retroarticular bone seen in other teleosts is absent in *Heteropneustes*.

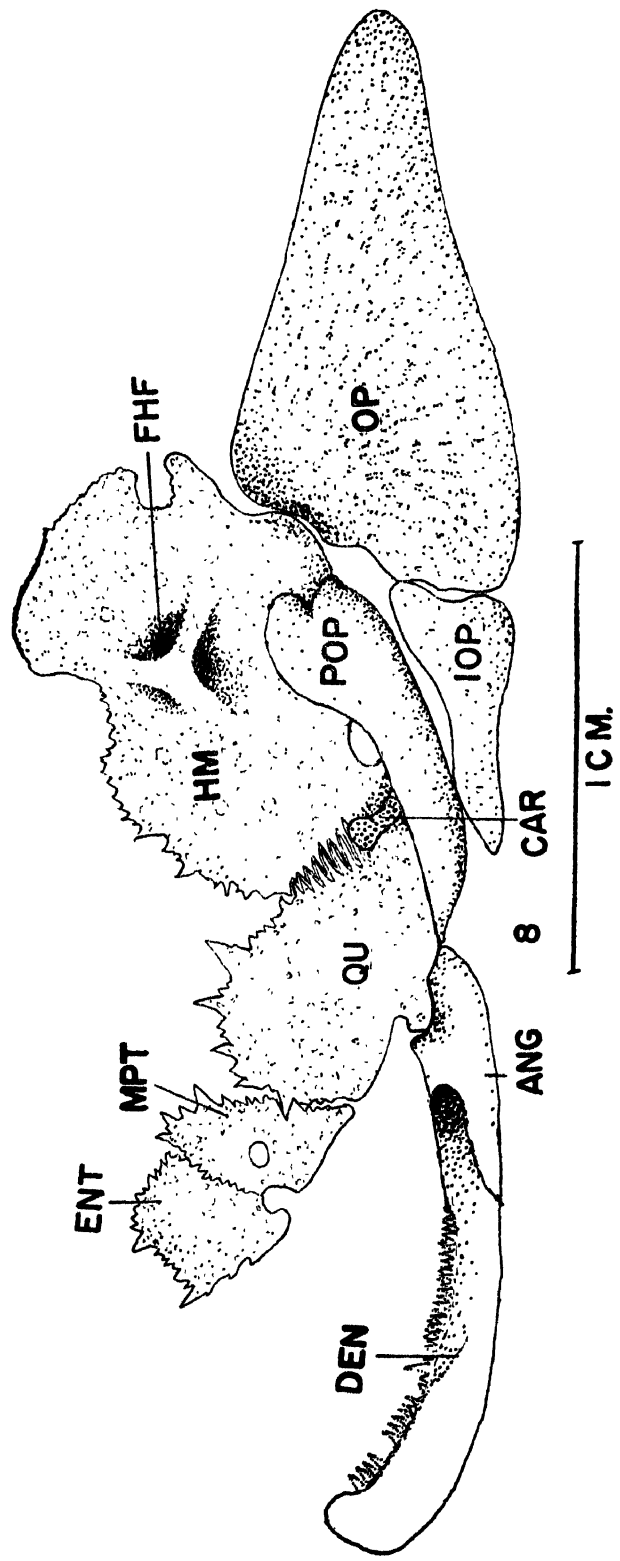
3. *The hyoid arch* : In the hyoid arch of the osteocranium, the hyomandibula which was originally fused with the quadrate in the chondrocranium, is ossified independently of the quadrate in the 33 mm. stage. The hyomandibular ossification in this stage is almost complete and possesses membranous extension (Text-fig. 5, MEH) dorsally and mesially. Ventrally the hyomandibula has a narrow process by which it articulates with the opercle bone (Text-fig., 5, 8, OP). The hyomandibula in the adult skull is a large irregular bone and articulates on the ventral surface of the sphenotic bone. It possesses laterally a number of ridges to which muscles are attached. Mesially a foramen for the exit of the hyomandibular branch of the facial nerve (Text-fig. 8, FHF) is noticed in the hyomandibula.



Text-fig. 6. Dorsal view of the adult skull of *Heteropneustes fossilis* (Bloch).

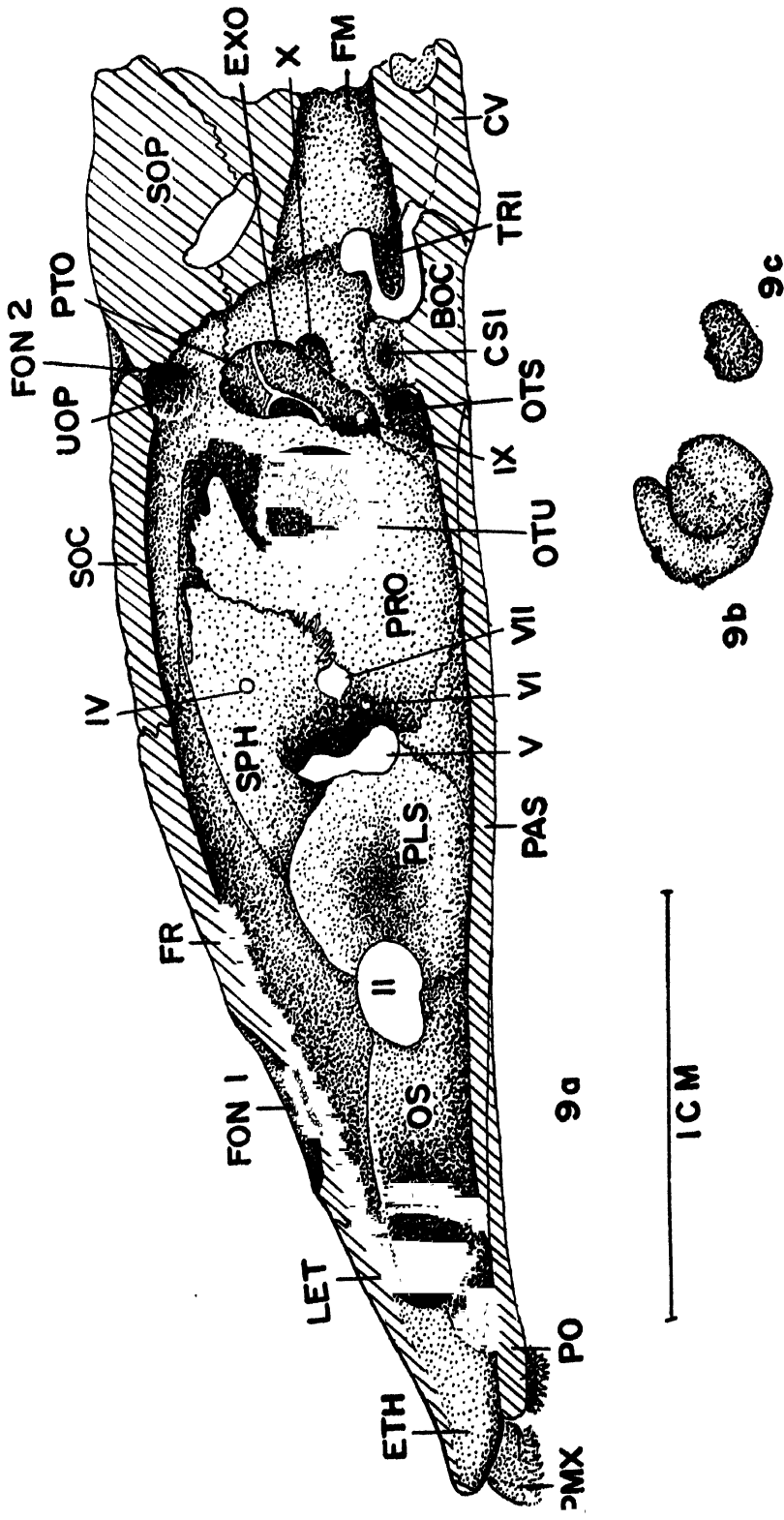


Text-fig. 7. Ventral view of the same.



Text-fig. 8. Lateral view of the upper and lower jaws with the opercular bones of the adult skull of *Heteropneustes tes fossilis* (Bloch).

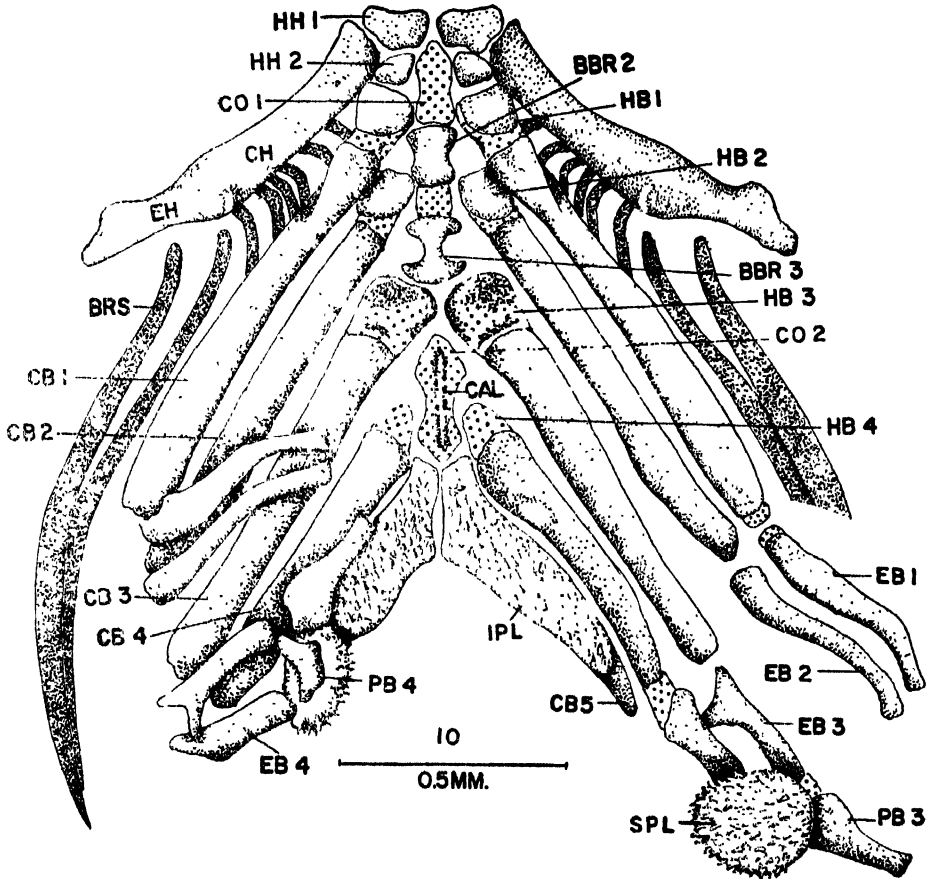




Text-fig. 9a. Sagittal sectional view of the adult skull of *Heteropneustes fossilis* (Bloch).  
9b. Otolith of the utricle. 9c. Otolith of the sacculus.

It is immovably connected anteriorly with the quadrate by a piece of cartilage and also by sutures and along the ventral border, the preopercle bone is firmly attached to it. A symplectic bone is absent.

The hyoid cornu of the adult skull consists of two pieces of hypohyals (Text-figs. 10, 11, HH1, HH2), ceratohyal (CH), an epihyal (EP) and a parahyoid (Text-fig. 11, PHY) or urohyal on the mid-ventral side of the two rami of the hyoid cornua. Closely associated with hyoid cornua are the branchiostegal rays (BRS).



Text-fig. 10. Dorsal aspect of the hyobranchial apparatus of the adult *Heteropneustes fossilis* (Bloch). One side has been extended to show the structures clearly.

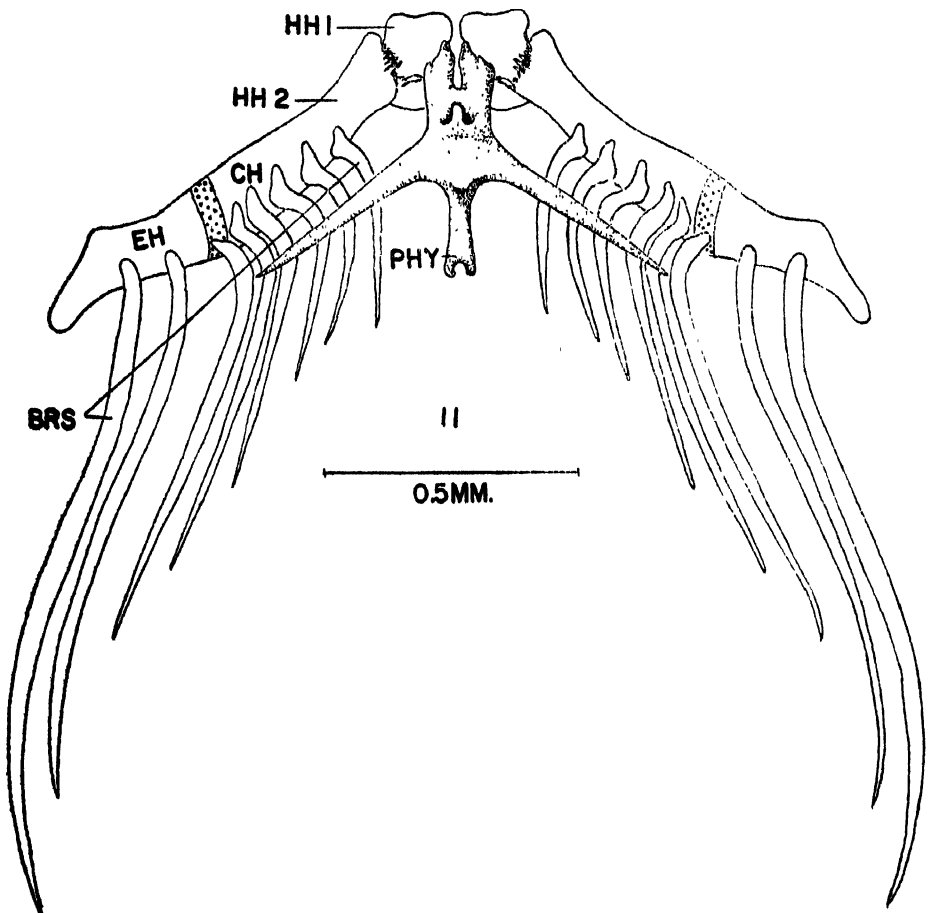
**The hypohyals :** The hypohyals on each side consist of the hypohyal anterior and hypohyal posterior, which are separated by a piece of unossified cartilage. The hypohyals are connected by ligaments with the corresponding bones of the other side. The anterior hypohyal is a stout piece, connected ventrally with the ceratohyal by means of sutures. The posterior hypohyal is a smaller triangular piece and lies close to the lower side of the ceratohyal. The two hypohyals are ossifications in the case of the original hypohyal cartilage which are not demarcated from the ceratohyal in the chondrocranium.

**The ceratohyals :** Each ceratohyal is a long bone showing a ridge on the ventral side and is flattened laterally. Anteriorly the ceratohyal is suturally connected with the hypohyal anterior and posteriorly it is connected with the epihyal by a thick piece of cartilage. On the ventral side the bone is slightly flat and gives attachment to the five branchiostegal rays.

*The epihyals* : Each epihyal is an ossification in the posterior end of the ceratohyal cartilage. The bone is a short piece, thick anteriorly and tapering posteriorly. The dorsal half of the epihyal is continuous with the ceratohyal and it is only in the lower half of the dorsal and ventral sides, the bone is separated from the ceratohyal by cartilage. The tapering posterior end of the epihyal is connected by a tiny piece of cartilage with the hyomandibula. The ventral side of the epihyal gives attachment to the two posterior branchiostegal rays.

A bony interhyal is absent. In the chondrocranium (Srinivasachar, 1957b) the interhyal which is continuous with the ventral side of the hyomandibula, connects the ceratohyal. In the ossified skull the interhyal continues to remain cartilaginous connecting the epihyal with the hyomandibula.

*The parahyoid* : The parahyoid (urohyal) bone is situated in the midventral line of the hyoid cornua between the hypophyals of the two sides. The parahyoid has no cartilaginous predecessor in the chondrocranium. It is a peculiarly-shaped bone connected on the ventral side to the hypophyals by means of ligaments. The bone has four rays (arms), an anterior, two lateral and a posterior arms. The anterior arm is stout having deep notch and the two portions of it are attached to the ventral side of the anterior hypophyals. The two lateral arms are fairly long pointed ones and extend ventrally obliquely on the branchiostegal rays attached to the ceratohyal. The posterior arm is a narrow portion and is bifid posteriorly-



Text-fig. 11. Ventral aspect of the hyoid cornua and the parahyoid of the adult *Heteropneustes fossilis* (Bloch).

*The branchiostegal rays :* There are altogether eight pairs of branchiostegals which are long pointed rays attached to the ventral surface of each hyoid cornua. These branchiosts gradually increase in length from the anterior to the posterior end. The anterior branchiostegal ray is the smallest and the posterior one is the longest of the eight rays. The anterior five branchiostegal rays are attached to the ventral surface of the ceratohyal, and the sixth ray is attached to the cartilage separating the ceratohyal from the epihyal. But the seventh and the eighth rays are attached to the ventral side of the epihyal. The eighth ray which is the longest of the lot is in the form of a large flat bone attached to the mesial side of the opercle.

*The branchial arches :* There are five branchial arches supporting the gills. The branchial arches are not completely ossified, but the cartilage of the chondrocranium persists in several regions. The median copula 1 (Text-fig. 10, CO1) of the chondrocranium (Srinivasachar, 1957b) shows two pieces of ossification representing the basibranchs, one of them between the first and second branchial arches and the other between the second and third. The rest of the portions of the original copula 1 and copula 2 (CO2) of the chondrocranium persists as unossified cartilages. The first two arches are not complete as they do not possess pharyngobranchs but the third and fourth arches are also incomplete in the absence of completely ossified hypobranchs. The fifth arch as in other teleosts is represented by ceratobranchs with patches of inferior pharyngeal teeth (IPL). In connection with the pharyngobranch of the third and fourth arches oval dentigerous patch, —the superior pharyngeal teeth (SPL) is noticed.

*The basibranchs :* The anterior portion of the original copula 1 of the chondrocranium persists as short piece of cartilage between the posterior ends of the hyoid cornua and the first branchial arches. The remaining portion of the copula is ossified into two pieces of bones, which may be labelled as basibranch 2 and 3 (Text-fig. 10, BBR2, BBR3) in order to differentiate between the cartilaginous and ossified portions. It may be assumed that the anterior cartilaginous piece or the copula 1 represents the fused basihyal and the first basibranch. The ossified copula represents the second basibranch. A small piece of original cartilage can be made out between the second and third basibranchs. The third basibranch is a small hour-glass shaped bone and the third branchial arch arises from the posterior end of this basibranch. Posteriorly in line with the third basibranch is a piece of cartilage biconcave laterally and is related to the fourth branchial arch. This piece of cartilage is the unossified basibranch of the fourth arch and represents the second copula (CO2) of the chondrocranium. Slight ossification (CAL) can be made out on the ventral compressed side of the cartilaginous second copula.

*The hypobranchs :* (Text-fig. 10, HB1-HB4) The hypobranchs of the first and second branchial arches are triangular pieces of bones connected with the respective ceratobranchs by the reminiscent cartilage. The first hypobranch is connected with the posterior end of the cartilaginous first copula, whereas the second hypobranch is at the postero-lateral end of the ossified second basibranch. The hypobranchs of the third arch are large flat pieces of cartilages showing slight ossifications in them. The cartilaginous hypobranchs extend inwards behind the third basibranch. The fourth hypobranch is probably represented as a cartilaginous piece with no ossifications and continues posteriorly as the bony fourth ceratobranch. The hypobranch arises from the postero-lateral region of the second copula. The hypobranchs are not traceable in the fifth branchial arch.

*The ceratobranchs :* (Text-fig. 10, CB1-CB5) All the five branchial arches show well ossified ceratobranchs. The ceratobranchs of the first three branchial arches are fairly long slightly flattened bones, and are connected posteriorly with the respective epibranchs. The ceratobranchs of fourth and fifth arches are almost of equal length and the fourth ceratobranch is connected to its epibranch by a thick piece of cartilage. The fifth ceratobranch has a thick patch of villiform

teeth of uniform size on the dorsal aspect forming the inferior pharyngeal teeth. I have not been able to see the actual origin of the teeth. The fifth ceratobranchs are situated in the posterior end of the second copula.

*The epibranchs* : (Text-fig. 10, EB1-EB4) The first four branchial arches possess distinct ossified epibranchs. The epibranchs of the first two branchial arches are comparatively short bent rod-like bones placed at an angle of more or less  $60^\circ$  to the respective ceratobranchs. These epibranchs are connected by cartilage at the ceratobranchs end while the other end is free of cartilage. The third epibranch is a small cylindrical bone having a pair of processes posteriorly, the lower process extends over the posterior end of the fourth epibranch. The upper process is attached to the ceratobranch. The fourth epibranch is a slightly curved piece connected to its ceratobranch by a strip of cartilage. Anteriorly the third and fourth epibranchs are connected to their pharyngobranchs.

*The pharyngobranchs* : (Text-fig. 10, PB 3-4) The first two branchial arches do not have pharyngobranchs as noticed in the early development of the chondrocranium (Srinivasachar, 1957b). The third and fourth branchial arches possess distinct ossified pharyngobranchs. The third pharyngobranch is a club shaped bone; the swollen end of the bone is connected to the third epibranch by a piece of cartilage and the narrow portion extends anteriorly. The fourth pharyngobranch is a smaller bone situated below the third pharyngobranch. In the chondrocranium the third and fourth pharyngobranchs are represented by a fused piece of cartilage.

A large oval dentigerous plate, the superior pharyngeal plate is noticed on the ventral surface of the third and fourth pharyngobranchs. The dentigerous plate appears to develop independently to the pharyngobranchs. The pharyngeal plate bears uniform pointed teeth and the plate extends over the epibranchs of the third and fourth branchial arches.

The order of appearance of bones in the osteocranium of *Heteropneustes* is as follows. The bones have been arranged according to their origin (cartilage or membrane bones).

| Stage                             | Type of ossifications  |  |
|-----------------------------------|--|--|
|                                   | Perichondral ossification<br>(Cartilago bones)                                     | Mombranous ossification<br>(Membrane bones)                                |
| 8 mm. stage                       |  | Maxilla.   |
| 12 mm. stage                      |  | Premaxilla.  |
| 19 mm. stage                      | Supraoccipital, Prootic, Basiooccipital, Quadrate.                                 | Parasphenoid, Frontal*, Angular*, Dentary*.                                |
| 21 mm. stage                      | Exoccipital, Lateral othmoid, Sphenotic*, Pterotic with supra-temporal extension*. | Posttemporal*.   |
| 29 mm. stage                      | Ethmoid.   |  |
| 33 mm. stage                      | Pleurospenoid, Orbitospenoid, Hyomandibula, Metapterygoid, Palatine.               | Prevomer, Supraorbital*, Nasal*, Preopercle*, Interopercle, Entopterygoid. |
| 49 mm. stage and the adult skull. |  | Intertemporal*, Suborbitals*.  |

\* The bones develop in association with sensory canal.

## DISCUSSION

The skull of *Heteropneustes* as in other catfishes (Gregory, 1933) shows a number of specialised features. The roof of the skull is flattened forming a cephalic shield which is actually formed by the large supraorbitals, intertemporals, pterotics and supratemporals. The skull is further broadened in *Heterobranchus* (Gregory, 1933) and *Clarias* (George, 1954).

In describing the bones of the ethmoid region of siluroid skull, the nomenclature employed is at variance. McMurich (1884) in describing the skull of *Amiurus* called the ethmoid bone a mesethmoid; later Kindred (1919) after studying the development of the bone in the same fish, employed the term supraethmoid as he was able to see a dermal and a perichondral ossification, following the nomenclature of Allis (1910) who described the bone in Loricati. Gregory (1933) in his figures of *Heterobranchus* and *Chrysichthys* has labelled the ethmoid bone as the dermethmoid and George (1954) has followed the same terminology in *Clarias*. In the development of *Heteropneustes*, I have observed that this bone arises completely as a perichondral ossification of the lamina precerebralis of the chondrocranium (Srinivasachar, 1957b) and it is only in later stages (33 mm. and 49 mm. stages) that this bone extends into the surrounding membrane. Hence I prefer to designate the bone merely an ethmoid as done by Goodrich (1909) and de Beer (1937). However, Bhimachar (1933) in describing the skull of some of the Indian catfishes has used the term supraethmoid. In the development of the osteocranium in *Heteropneustes*, it has been observed that except the palatine ossification all the other perichondral ossifications have membranous extensions into the surrounding membrane.

The other bones of the ethmoid region in *Heteropneustes* are the nasals, the lateral ethmoid and ventrally the median prevomer. The nasals develop around the anterior end of the sensory canal as narrow tubular bones. These bones resemble more the nasals of Cyprinoids (Ramaswami, 1955) than those of other siluroids studied. In *Heterobranchus* (Gregory, 1933) and *Clarias* (George, 1954) the nasals are small flat bones, but in *Silurus* (Goodrich, 1909) the nasals are large elongated plates. The lateral ethmoids are variously named in different groups of fishes. Sagemehl (1891) in Cyprinoids and Characinidae, Goodrich (1909) in *Clarias* and Gregory (1933) in *Heterobranchus* have termed the bone prefrontal and the same bone in *Amiurus* (Kindred, 1919) has been designated ectethmoid. In *Heteropneustes* the lateral ethmoid arises as a perichondral ossification in the lamina orbitonasalis of the chondrocranium and in the adult skull the bone has a large process ventrally which is connected with the antero-lateral extensions of the orbitosphenoid by a piece of cartilage. George (1954) does not report a connection of the lateral ethmoid with the orbitosphenoid in his species of *Clarias*.

The T-shaped prevomer carries two patches of teeth separated by a wide gap in *Heteropneustes*, whereas in *Clarias* (George, 1954) the bone designated as vomer is a crescent shaped one having a large patch of teeth ventrally.

The pleurosphenoids are well developed bones in *Heteropneustes* occupying the position between the optic and trigeminal foramina. These bones are seen to develop partly as perichondral ossification in the orbital cartilage and partly in membrane. Kindred (1919) has referred to this bone as alisphenoid in *Amiurus* and Bhimachar (1933) also has adopted the same nomenclature in describing the Indian catfishes. Both Goodrich (1930) and de Beer (1937) having considered the true nature of the alisphenoid have come to the conclusion that this should not be called alisphenoid in the vertebrates lower than mammals. A pleurosphenoid has not been described in *Clarias* (George, 1954).

Kindred (1919) and Bhimachar (1933) have recognised a suprasphenoid (basisphenoid) being fused with parasphenoid dorsally in *Amiurus* and other siluroids.

Such a bone is not observed in the development of skull of *Heteropneustes* and also it is absent in *Clarias* (George, 1954).

The occurrence of dorsal fontanelles between the frontals and in the supra-occipital appears to be a common feature noticed in the skull of Siluroidea. David (1935) has used the size of these fontanelles in the classification of the members of Clariidae. In *Galeichthys* and *Bagre*, Merriman (1940) described that the fontanelle might probably be meant for the passage of sensory nerves to snout and barbels. In *Heteropneustes* no nerves are seen to pass through the anterior fontanelle and it is covered by a membrane in the adult skull. These fontanelles appear to represent the remnants of the original large fontanelle of the chondrocranium, which has been reduced by the extensive growth of the frontals anteriorly and supra-occipital posteriorly. In *Clarias* (George, 1954) has also not been able to see any nerves passing through the fontanelle but has observed a foramen in the frontal bone through which passes a nerve to the skin. I have observed such foramina in the frontals on either side of the anterior fontanelle for the exit of nerves to the skin in *Clarias batrachus* which I have examined for the sake of comparison. The occurrence of frontoparietal fontanelles has been observed in large number of carp skulls like *Gobiobotia*, *Saurogobio*, *Pseudogobio* and *Abbottima* (Ramaswami, 1955), *Cyprinus carpio* (Sagemehl, 1891). Though Sagemehl (1891) did not attach much importance to the presence of the fontanelles Taranetz (1938) used the presence or absence of the fontanelles in the occipital region for dividing the gudgeons into groups. Ramaswami (1955) is of the opinion that in the bottom dwelling forms the fontanelles are necessary for some physiological functions.

There appears to be much confusion with regard to the nomenclature of bones of the otic region : the pterotic has been described by Kindred (1919) and Bhimachar (1933) as a squamoso-pterotic since the bone in *Amiurus* develops by a fusion of a perichondral and a membranous ossifications. But these ossifications in *Amiurus* appear to be fused from the start and the membranous part of the pterotic bone is the supratemporal which is a lateral line canal component subsequently fusing with the primary ossification. In *Heteropneustes* also I have been able to see that the membranous part (supratemporal) and the perichondral part of pterotic arises as a single ossification from the start : I have therefore called it merely pterotic. Bhimachar (1933), however, has noticed the presence of separate supratemporal in *Rita*, *Pangasius*, *Macrones*, *Silundia* and *Plotosus*. In *Esoxocetus* (Lasdin, 1913) the supratemporal and pterotic develop separately and remain separate as distinct bones, but in *Salmo* (de Beer, 1937) the two bones arise separately but fuse later.

The sphenotic has been referred to by Cuvier (1826) and Parker (1872) as postfrontal homologous to the similar bones in reptiles. Ridewood (1904) also retained the same name in his studies and similarly Goodrich (1909) has followed the same nomenclature in labelling the sphenotics of *Clarias*. It has been observed in *Heteropneustes* that the sphenotic is an ossification in the region of the anterior semicircular canal of the auditory capsule where the articulation of hyomandibula is seen. The intertemporal bone which arises as a membrane bone outside the auditory capsule in *Heteropneustes* and also probably in *Clarias*. It has been described as dermosphenotic by George (1954) and in *Heterobranchus* by Gregory (1933). But de Beer (1937) is of the opinion that "there is no reason to think that the postfrontal ever was the 'dermal representative' of the sphenotic ; the postfrontal is the uppermost bone of the postorbital series, and it is the intertemporal which comes in question as the dermal representative of the sphenotic (p. 498)". It is known that in *Dactylopterus* (Allis, 1910) and *Polypterus* (Allis, 1922) the postfrontal is fused with the sphenotic. In *Osteolepis* (Goodrich, 1930) the postfrontal, intertemporal and supra-temporal are separately present. Therefore, I have used the terms sphenotic and pterotic in describing the cartilage bones of the otic region of fishes and the terms intertemporal and supratemporal for the membrane

ossification which have been referred to by some as dermsphenotic and dermpterotic (squamosal) respectively.

It has been generally observed that the parietal bones are absent in all the siluroids studied. In this connection some authors (Goodrich, 1909 ; de Beer, 1937) have doubted whether the parietals have fused with the supraoccipital. But a study of the development of skull in *Heteropneustes* has revealed that the supraoccipital which arises as a perichondral ossification in the region of tecti synoticum and posteriorly of the chondrocranium, extends into the membrane both anteriorly and posteriorly, and separate parietal ossification is never observed. It is, therefore, concluded that the supraoccipital alone is developed and it extends to the region of the parietals also.

A supraoccipital spine is considerably elongated and well developed in *Heteropneustes* and in *Clarias* (George, 1954) the spine is almost absent. The supraorbital is a well developed bone in *Heteropneustes* as in *Heterobranchus* (Gregory, 1933) and *Clarias* (George, 1954).

The metapterygoid is seen to develop from the processus pterygoideus of the quadrate of the chondrocranium in *Heteropneustes* and closely attached to the metapterygoid is another membrane bone which is designated here as entopterygoid. A pterygoid (ectopterygoid) is absent. Regan (1911) opined that the metapterygoid in the Ostariophysi had moved over the top of the quadrate and taken the place of the pterygoid, and therefore, the pterygoid was absent, except in Bagridae where it formed a small plate behind the palatine. David (1935) also reports only two pterygoid bones in the members of Clariidae. Merriman (1940) in *Galleichthys* and Bagre and George (1954) in *Clarias* have reported the presence of pterygoid lying dorsal or lateral to the metapterygoid respectively. But in *Clarias batrachus* examined by me all the three the metapterygoid, entopterygoid and the ectopterygoid bones are present ; the ectopterygoid is a small bone.

In *Heteropneustes* the palatines are completely perichondral ossifications without any membranous extension and remain independent of the metapterygoid, as observed in the development of the chondrocranium (Srinivasachar, 1957c). Similar palatines have been observed in other siluroids like *Heterobranchus* (Gregory, 1933) and *Clarias* (George, 1954). The independent nature of the palatines have been noticed even in the development of the chondrocranium in *Heteropneustes* and *Clarias* where the pterygoid process chondrifies independently and does not fuse with the quadrate at any time of the development. This condition has been observed by me (Srinivasachar, 1956b, 1957b, 1957c) in all the siluroids studied except *Arius* where the pterygoid process is fused with the quadrate.

In *Heteropneustes* and *Clarias* the pterygoid bones are firmly attached with the prevomers by means of tendons and connective tissue. But in *Batasio* (Srinivasachar, 1956a) the upper jaw possesses an additional articulation of pterygoid with the orbitosphenoid bone in addition to the usual articulation with the hyomandibula. Such a condition has not been noted in any other siluroid studied.

In the lower jaw, only two bones, the angular and dentary are developed around Meckel's cartilage in *Heteropneustes* and the splenial reported in *Clarias* by George (1954) is absent. I have also not seen a splenial in *Clarias batrachus* which I have examined for the sake of comparison. However, Haines (1937) has observed the invasion of the angular ossification in Meckel's cartilage in *Mugil*, *Sardina* and *Trigla* and also the presence of an endochondral ossification in the processus retroarticularis of Meckel's cartilage. In *Heteropneustes* both angular and dentary develop outside Meckel's cartilage and a retroarticular bone is completely absent. In *Ophicephalus* (Srinivasachar, 1955) a retroarticular is seen ossifying in the retroarticular process of Meckel's cartilage.

The branchial arches of *Heteropneustes* differ considerably from the similar arches of *Clarias* (George, 1954). In *Heteropneustes* the hypobranch of the third arch is a large piece of cartilage where slight ossification has been noticed and the



hypobranch of the fourth arch is also distinct and is not ossified. But in *Clarias* (George, 1954) the hypobranchs are absent in both the third and fourth branchial arches; and a large triangular cartilaginous plate to which the ceratobranchs of all the arches are connected, has been described by him. I have not been able to see such a structure either in the chondrocranium of *Clarias batrachus* (Srinivasachar, 1957b) or in the adult skull of the same that I have examined. In the chondrocranium of *Clarias* the median copula is represented by two pieces of cylindrical cartilages to which the hypobranchs are attached and not to the ceratobranchs as shown by George (1954) in the adult skull of *Clarias lazera*.

The opercular bones of *Heteropneustes* are reduced as in other siluroids (Gregory, 1933). The opercle bone is larger than in *Clarias*. The preopercle is firmly attached to the ventral edge of the hyomandibula and the quadrate in *Heteropneustes* and a subopercle is absent as in other siluroids.

The following table gives the synonymy of the terms employed by various authors in describing the skull of catfishes.

TABLE 1

| Nomenclature followed in <i>Heteropneustes</i> following that of de Beer (1937) | Goodrich (1909)     | Kindred (1919)     | Gregory (1933) | Bhimachar (1933)   | George (1954)    |
|---|---------------------|--------------------|----------------|--------------------|------------------|
| Ethmoid   | Ethmoid             | Supraethmoid       | Dermethmoid    | Supraethmoid       | Dermethmoid      |
| Lateral ethmoid.  | Prefrontal          | Ectethmoid         | Prefrontal     | Ectethmoid         | Lateral-ethmoid. |
| Prevomer  | —                   | Vomer              | Vomer          | Vomer              | Vomer            |
| Pleurospenoid   | —                   | Alisphenoid        | Alisphenoid    | Alisphenoid        | —                |
| Pterotic  | Pterotic            | Squamoso-pterotic. | Pterotic       | Squamoso-pterotic. | Pterotic         |
| Sphenotic   | Postfrontal         | Sphenotic          | Sphenotic      | Sphenotic          | Sphenotic        |
| Intertemporal   | Lateral cheek bone. | —                  | Dermisphenotic | —                  | Dermisphenotic   |
| Antorbital  | Suborbital          | Suborbital         | Adnasal        | Infraorbital       | Minor maxillary. |
| Hypohyal  | —                   | —                  | —              | —                  | Glossohyal       |

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## LIST OF ABBREVIATIONS

- ANG : Angular.  
 AOR : Antorbital.  
 BBR 2 : Basibranchial 2.  
 BBR 3 : Basibranchial 3.  
 BOC : Basisoccipital.  
 BR5 : Branchiostegal rays.  
 CAL : Ossifications.  
 CB 1-5 : Ceratobranchs 1 to 5.  
 CH : Ceratohyal.  
 CO 1-2 : Cartilaginous copula 1 and 2.  
 COM : Commissural sensory canal.

|          |   |
|----------|---|
| CSI :    | Cavum sinus impar.  |
| CV :     | Complex vertebrae.  |
| DEN :    | Dentary.  |
| EB 1-4 : | Epibranchs 1 to 4.  |
| EH :     | Epihyal.  |
| ENT :    | Entopterygoid.  |
| ETC :    | Ethmoid cornu.  |
| ETH :    | Ethmoid.  |
| EXO :    | Exoccipital.  |
| FHF :    | Foramen for the hyomandibular branch of the facial nerve. |
| FM :     | Foramen magnum.   |
| FON 1 :  | Anterior fontanel in the Frontals.                        |
| FON 2 :  | Posterior fontanel in the Supraoccipital.                 |
| FR :     | Frontal.  |
| HB 1-4 : | Hypobranchs 1-4.  |
| HH 1 :   | Hypohyal anterior.  |
| HH 2 :   | Hypohyal posterior.                                       |
| HM :     | Hyomandibula.   |
| IOP :    | Interopercle.   |
| IPL :    | Inferior pharyngeal plate.                                |
| LET :    | Lateral ethmoid.  |
| LAC :    | Lacrimonal.   |
| MAX :    | Maxilla.  |
| MEE :    | Membranous extension of the ethmoid bone.                 |
| MEH :    | Membranous extension of the hyomandibula.                 |
| MEP :    | Membranous extension of the metapterygoid.                |
| MEQ :    | Membranous extension of the quadrate.                     |
| MPT :    | Metapterygoid.  |
| NS :     | Nasal.  |
| OP :     | Opercle.  |
| OS :     | Orbitosphenoid.   |
| OT :     | Region of the otolith of utricle in prootic bone.         |
| OTS :    | Cavum sacculus.   |
| OTU :    | Cavum utriculus.  |
| PAL :    | Palatine.   |
| PAS :    | Parasphenoid.   |
| PB 3 :   | Pharyngobranch 3.   |
| PB 4 :   | Pharyngobranch 4.   |
| PHY :    | Parahyoid.  |
| PLS :    | Pleurosphenoid.   |
| PMS :    | Preopercle mandibular sensory canal.                      |
| PO :     | Preopercle.   |
| POP :    | Preopercle.   |
| PRO :    | Prootic.  |
| PTM :    | Posttemporal.   |
| PTO :    | Pterotic.   |
| QU :     | Quadrate.   |
| SC :     | Suborbital sensory canal.                                 |
| SCO :    | Sensory canal ossicle.                                    |
| SO 1-2 : | Suborbitals 1 and 2.                                      |
| SOC :    | Supraoccipital.   |
| SOP :    | Supraoccipital spine.                                     |
| SOS :    | Supraorbital sensory canal.                               |
| SPH :    | Sphenotic.  |
| SPL :    | Superior pharyngeal plate.                                |
| SUO :    | Supraorbital.   |
| TRI :    | Tripterus.  |
| TSC :    | Temporal sensory canal.                                   |
| UOP :    | Upper opening of the posterior semicircular canal.        |
| II :     | Optic foramen.  |
| IV :     | Trochlear foramen.  |
| V :      | Trigeminal foramen.                                       |
| VI :     | Abducens foramen.   |
| VII :    | Facial foramen.   |
| IX :     | Glossopharyngeal foramen.                                 |
| X :      | Vagus foramen.  |

# HISTOCHEMICAL AND HISTOLOGICAL STUDIES IN NORMAL AND FOLIC ACID AND VITAMIN B<sub>12</sub> DEFICIENT RATS

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## ABSTRACT

The distribution of alkaline and acid phosphatases, RNA and DNA was studied by histochemical technic in normal and vitamin B<sub>12</sub> and folic acid deficient rats. Tissues were also stained with haematoxylin and eosin for histological study.

The alkaline phosphatase was increased in kidney, adrenals, spleen, testes, and thyroid in all animals made deficient in the two vitamins. In the deficient animals the acid phosphatase activity increased in liver and adrenal, and disappeared completely in the pituitary.

The vitamin B<sub>12</sub> and folic acid deficient rats showed diminished RNA contents in liver, pancreas, adrenals, spleen, and testes ; diminished DNA contents in pancreas, adrenals and testes ; and increased DNA content in the spleen.

The deficient animals showed marked histological changes in liver, thyroid, testes, and spleen.

## INTRODUCTION

Reproductive failure in vitamin B<sub>12</sub> deficient rats, and gross underdevelopment in various tissues of newborn rats from vitamin B<sub>12</sub> deficient mothers have been reported by Dryden, Hartman and Cary (1951) ; Lepkovsky *et al.* (1951) ; Richardson, Witten and Couch (1951), and Jones, Brown, Richardson and Sinclair (1955). Ferguson and Couch (1954) observed degenerative changes in heart, liver, thyroid, and kidney in chick embryos from hens deficient in vitamin B<sub>12</sub>.

Sebrell and Harris (1954) have cited several references to implicate vitamin B<sub>12</sub> in the process of nucleic acid synthesis. Stern, Taylor and Russell (1949) have reported a diminution in liver cytoplasmic basophilia in vitamin B<sub>12</sub> deficient rats. Dempsey and Wislocki (1946) have pointed out that basophilia, shown to be ribonucleoprotein, are intimately related to protein synthesis. Hence in vitamin B<sub>12</sub> deficient animals formation of nucleoprotein through the nucleoside is limited, and consequently protein synthesis in the body would be retarded. Rasch *et al.* (1955) have reported a diminution in liver nucleic acids in vitamin B<sub>12</sub> deficiency. Rose and Schweigert (1952) have observed degenerative changes in testes and thyroid in vitamin B<sub>12</sub> deficient rats. Biochemical studies of liver (Rose and Schweigert, 1952 ; Schweigert, Scheid and Downing, 1954) have shown both DNA and RNA to be decreased per gram of liver in vitamin B<sub>12</sub> deficient rats. Reports of tissue abnormalities of folic acid deficiency have not been reported so far.

It is presumed that phosphatases play an exceedingly important rôle in the metabolism of carbohydrates, lipids, and nucleoproteins. Colowick *et al.* (1947) have reported that certain hormones (e.g., of adrenal cortex, pituitary, and pancreas) act on hexokinase, which are very closely related to phosphatases. Kellerman (1955) has indicated a rôle of phosphatases in dephosphorylation, and now it is understood that phosphatases act as catalyst in the process of dephosphorylation of certain phosphate esters. Possible relationship of phosphatases to various hormones is also indicated by Dempsey, Greep and Dean (1949) and Mathies, Goodman and Palm (1952).

Ling and Chow (1952, 1953, 1954) have presented evidences to show that vitamin B<sub>12</sub> deficiency causes derangements in carbohydrate and lipid metabolism. Williams, Nichol and Elvehjem (1949) have indicated the rôle of folic acid in carbohydrate metabolism.

The present work deals with the studies on the distribution of alkaline and acid phosphatases, ribonucleic acid, and desoxyribonucleic acid in the various tissues of normal and folic acid and vitamin B<sub>12</sub> deficient rats. Histological studies of the tissues of these animals were also undertaken.

### MATERIALS AND METHODS

Weanling male rats weighing between 30 and 35 gm. were divided into two groups with approximately the same weights. They were fed *ad-libitum*, a purified vitamin B<sub>12</sub> and folic acid free diet (Fatherpaker *et al.*, 1955). Casein was made vitamin free by hot alcohol extraction. Microbiological tests showed no detectable amounts of the two vitamins in the casein preparation used. The salt mixture No. 2 (U.S.P.) was finely powdered and mixed uniformly with the diet. Rats receiving the folic acid and vitamin B<sub>12</sub> free purified diet with an oral supplement of 0.5 µg of vitamin B<sub>12</sub> and 8 µg of folic acid per animal per day, were taken as normal controls. The experimental group received 0.3 per cent of a preparation of iodinated casein and sulphasuxidine at a level of 1 per cent in the diet itself, without any supplement of folic acid and vitamin B<sub>12</sub>. After about 3-4 weeks, the animals without folic acid and vitamin B<sub>12</sub> supplements, started losing weight. The animals were killed by decapitation after about five weeks on the experimental diet, when they were deficient with regard to the above vitamins as revealed by hematological studies and microbiological assay of the vitamins in the liver. The studies were conducted with six normal and six deficient animals.

The tissues were immediately removed, cut into 2-3 mm. thick pieces and fixed overnight in chilled acetone, chilled 80 per cent alcohol, 10 per cent neutral formalin, and Zenker-formol. They were then dehydrated and embedded in paraffin. Alkaline phosphatase content of the acetone fixed tissues were measured by the histochemical method of Gomori (1946). Acetone fixed tissues were also used for the estimation of acid phosphatase by the modified histochemical method of Gomori (1950). DNA and RNA in alcohol and neutral formalin fixed tissues were measured by the method of Kurnick (1952). Feulgen reaction (Coleman, 1938; Stowell, 1945) for DNA were also carried out in the alcohol and neutral formalin fixed tissues. Zenker-formol fixed tissues were used for the histological study.

The following tissues were studied: liver, kidney, adrenal, spleen, pancreas, testes, thyroid, and pituitary.

### RESULTS

In the different groups of animals studied, changes observed did not vary from animal to animal. The results of the investigations carried out are summarized in Table I.

#### *Alkaline and Acid Phosphatases*

**Liver:** Very little alkaline phosphatase was found to be present in the normal liver and no significant change could be observed between the liver of normal and of 'deficient' animals.

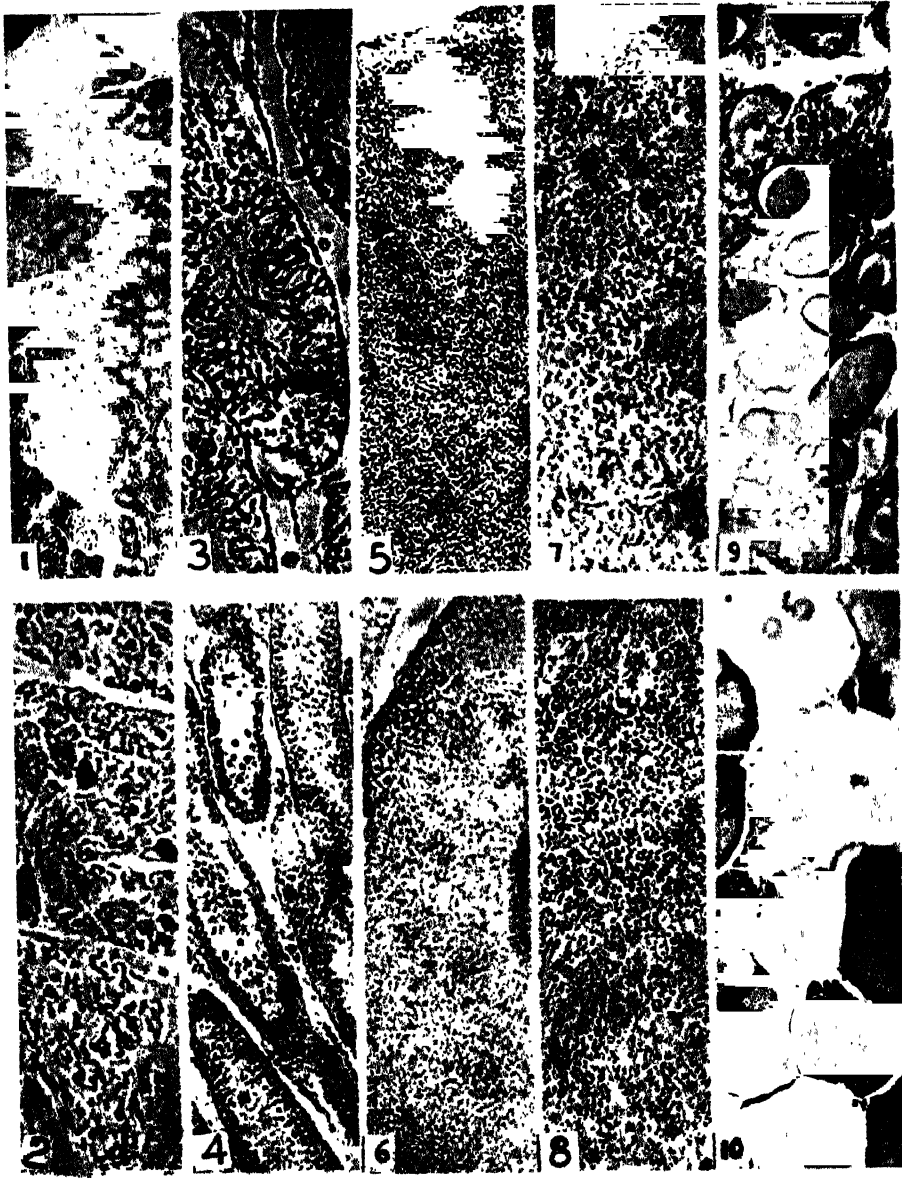
The acid phosphatase gave an intense reaction in the hepatic cells and it seems to have been increased slightly in the liver of 'deficient' animals.



1. Testes of normal rat showing distribution of alkaline phosphatase.  $\times 110$ .
2. Testes of deficient rat showing distribution of alkaline phosphatase.  $\times 110$ .
3. Thyroid of normal rat showing distribution of alkaline phosphatase.  $\times 220$ .
4. Thyroid of deficient rat showing distribution of alkaline phosphatase.  $\times 220$ .
5. Adrenal cortex of normal rat showing distribution of alkaline phosphatase.  $\times 110$ .
6. Adrenal cortex of deficient rat showing distribution of alkaline phosphatase.  $\times 110$ .
7. Kidney of normal rat showing distribution of alkaline phosphatase.  $\times 110$ .
8. Kidney of deficient rat showing distribution of alkaline phosphatase.  $\times 110$ .
9. Anterior pituitary of normal rat showing distribution of acid phosphatase.  $\times 110$ .
10. Anterior pituitary of deficient rat showing a negative reaction for acid phosphatase.  $\times 110$ .



1. Liver of normal rat showing distribution of acid phosphatase. 110.
2. Liver of deficient rat showing distribution of acid phosphatase. 110.
3. Liver of normal rat showing concentration of RNA. 110.
4. Liver of deficient rat showing concentration of RNA. 110.
5. Pancreas of normal rat showing concentration of RNA. 110.
6. Pancreas of deficient rat showing concentration of RNA. 110.
7. Testes of normal rat showing concentration of RNA. 110.
8. Testes of deficient rat showing concentration of RNA. 110.
9. Adrenal cortex of normal rat showing concentration of RNA. 110.
10. Adrenal cortex of deficient rat showing concentration of RNA. 110.



1. Pancreas of normal rat showing concentration of DNA. 110.
2. Pancreas of deficient rat showing concentration of DNA. 110.
3. Testes of normal rat showing concentration of DNA. 110.
4. Testes of deficient rat showing concentration of DNA. 110.
5. Adrenal cortex of normal rat showing concentration of DNA. 110.
6. Adrenal cortex of deficient rat showing concentration of DNA. 110.
7. Spleen of normal rat showing concentration of DNA. 110.
8. Spleen of deficient rat showing concentration of DNA. 110.
9. Thyroid of normal rat. 220.
10. Thyroid of deficient rat showing enlarged follicles and flat secretory cells. 220.





*Kidney*: The maximum amount of alkaline phosphatase was located in the brush border of the kidney tubules. Considerable amount of the enzyme was present in the kidney cortex evenly distributed throughout, except that in the glomeruli it was much less. An increase, more pronounced in the brush border of the tubules, was observed in the kidney of the deficient animals.

The acid phosphatase showed an even distribution and no change could be observed between the kidney of normal and of deficient animals.

TABLE I

*Distribution of alkaline and acid phosphatases, RNA and DNA in the tissues of normal rats, (N) and rats deficient (D) in vitamin B<sub>12</sub> and folic acid*

| Tissues   | Alkaline Phosphatase |      | Acid Phosphatase |     | RNA |     | DNA |     |
|-----------|----------------------|------|------------------|-----|-----|-----|-----|-----|
|           | (N)                  | (D)  | (N)              | (D) | (N) | (D) | (N) | (D) |
| Liver     | +                    | +    | ++               | +++ | ++  | +   | ++  | +   |
| Kidney    | ++                   | ++++ | ++               | ++  | ++  | ++  | ++  | ++  |
| Adrenal   | ++                   | ++++ | ++               | ++  | ++  | +   | ++  | +   |
| Spleen    | ++                   | +++  | ++               | ++  | ++  | +   | ++  | +++ |
| Pancreas  | ++                   | ++   | ++               | ++  | ++  | +   | ++  | +   |
| Testes    | ++                   | ++++ | —                | —   | ++  | +   | ++  | +   |
| Pituitary | ++                   | ++   | ++               | —   | ++  | ++  | ++  | ++  |
| Thyroid   | ++                   | ++++ | ++               | ++  | ++  | ++  | ++  | ++  |

*Adrenals*: The glomerular zone of the adrenal cortex showed the most intense activity for alkaline phosphatase. The intensity of the enzyme activity was found to be in a descending order in zona fasciculata and zona reticularis respectively. The adrenal medulla contained minimal amounts of alkaline phosphatase. In the adrenal of deficient animals the enzyme was found to be considerably increased, and though the distribution appeared to be on a similar pattern as in the normal adrenal, the increase was more pronounced in the zona glomerulosa and zona fasciculata.

The intensity of acid phosphatase was found to be more or less the same in the adrenals of both normal and deficient animals, though probably, there is a very slight increase in the adrenals of deficient animals.

*Spleen*: Alkaline phosphatase was evenly distributed in the spleen pulp. Minimal activity was observed in the Malpighian corpuscles. In the spleen of deficient animals the alkaline phosphatase activity was found to be increased to some extent.

No change was perceptible in the acid phosphatase activity in the spleen of normal and deficient group of animals.

*Pancreas*: Pancreas gave a positive reaction for alkaline phosphatase. The activity in the acinar cells is much more pronounced than in the islets. No change could be observed in the alkaline phosphatase activity in normal pancreas and in pancreas of deficient animals.

The acid phosphatase also did not show any difference in the normal pancreas and pancreas of deficient animals.

*Testes*: The alkaline phosphatase activity of the seminiferous tubules of the testes of deficient animals was considerably increased as compared with the normal controls.

The acid phosphatase did not show any variation in the normal and deficient testes. The normal testes gave a negative reaction for acid phosphatase, and in

the testes of deficient animals the activity could be located at some isolated spots, which does not seem to be significant.

*Pituitary* : There seems to be a selective distribution of both alkaline and acid phosphatases in the pituitary, which, possibly, may be due to the different cell types. The intensity of alkaline phosphatase is more pronounced in the posterior lobe of the pituitary, whereas the acid phosphatase is more distinctly distributed in the anterior lobe. The pars intermedia gives a diffuse reaction for both of these enzymes. No change could be observed in the alkaline phosphatase activity of normal pituitary and pituitary of deficient animals.

The pituitary of the deficient animals gives a more or less negative reaction for acid phosphatase, while the normal pituitary shows a pronounced activity of acid phosphatase.

*Thyroid* : The secretory cells of the thyroid give an intense reaction for alkaline phosphatase and it is considerably increased in the thyroid of deficient group of animals.

The acid phosphatase does not seem to indicate any perceptible variation.

#### *Ribonucleic Acid and Desoxyribonucleic Acid*

The RNA and DNA studied in all the above tissues, in general, seem to have been decreased significantly in the tissues of vitamin B<sub>12</sub> and folic acid deficient animals. The more perceptible changes were observed in liver, pancreas, adrenal, spleen, and testes which showed a decrease in RNA content in the tissues of the deficient group of animals.

Thyroid, pituitary, and kidney from normal animal and animals rendered deficient in vitamin B<sub>12</sub> and folic acid did not show any significant change in the RNA content.

The DNA was found to be decreased in pancreas, adrenal and testes. In the liver of deficient animals also the DNA seems to be slightly decreased though it does not seem to be very distinct.

Contrary to the above results, spleen of the deficient animals showed a marked increase in the DNA content as compared with the normal. The number of nuclei is greatly increased. Thyroid, pituitary and kidney did not show any variation as regards DNA content.

#### *Histological Study*

The haematoxylin and eosin stained sections on microscopic observation showed changes in liver, spleen, testes, and thyroid only. In the rest of the tissues no variations from the normal could be observed.

*Liver* : The clumped nature of the cytoplasmic basophilia of the normal liver was not retained in the liver of the deficient group of animals. The granules were evenly and scarcely distributed and a marked decrease in the cytoplasmic basophilia was observed, which is in agreement with the work of Stern, Taylor and Russel (1949). The liver showed heavy haemorrhage, and fat infiltration was also observed to a slight extent.

*Spleen* : In most of the animals rendered deficient in vitamin B<sub>12</sub> and folic acid the size of the spleen is significantly increased. The number of nuclei showed a marked increase in the deficient tissue which correspondingly increases the DNA content also.

*Testes* : Testes show gross degenerative changes in deficient tissues, which confirm the works reported by Rose and Schweigert (1952). The seminiferous tubules were markedly shrunken, thereby increasing the intertubular space. The interstitial cells show marked signs of degeneration. Spermatogenetic activity

is considerably reduced and only a few spermatogonia remained in the germinal epithelium.

*Thyroid*: The thyroid follicles of the deficient tissue were enlarged and were full of colloid, which takes a deep stain. There were very few or no vacuoles in the follicles.

The secretory cells lining the follicles were low cuboidal or flat. A marked reduction in the size of the secretory cells occur, the reduction being primarily in the cytoplasm of the cells. The gland as a whole was highly vascular. These changes in the thyroid were similar to those reported by Wang *et al.* (1954).

## DISCUSSION

There seems to be a general increase in the alkaline and acid phosphatase contents of the various tissues of rats made deficient in both folic acid and vitamin B<sub>12</sub>. The increase in alkaline phosphatase is more pronounced in kidney, adrenal, spleen, testes, and thyroid. The acid phosphatase is increased in liver and adrenals. There is total disappearance of acid phosphatase from the deficient pituitary.

Our knowledge concerning the biochemical function of phosphatase is still limited, though it is presumed to play an important rôle in the metabolism of carbohydrates, lipids, and nucleoproteins. The derangement in carbohydrate metabolism in vitamin B<sub>12</sub> and folic acid deficiencies have been reported by Ling and Chow (1952, 1953, 1954) and Williams, Nichol and Elvehjem (1949). The changes observed by us in the alkaline and acid phosphatase content of tissues in vitamin B<sub>12</sub> and folic acid deficiency may add to the evidence of the rôle of the two vitamins in carbohydrate metabolism. Kellerman's (1955) report on the rôle of phosphatase in dephosphorylation and the work of Piccardo and Salvetti (1955) and other workers, suggesting the close relationship between phosphate turnover and vitamin B<sub>12</sub>, seem to explain the changes in alkaline and acid phosphatase observed by us.

Moog (1946) and Dempsey and Wislocki (1946) have suggested that adrenal alkaline phosphatase plays an important rôle in the metabolism of lipids and so possibly in the synthesis of adrenal cortical hormones. Boxer *et al.* (1955) have observed a considerable increase in the coenzyme A contents of liver and kidney, which, in turn, may affect the Krebs's cycle. The considerable increase observed in the alkaline phosphatase content of the deficient adrenal may indicate a disturbed physiological function of the gland, which can be presumed from the reports on deranged lipid metabolism and the changes in coenzyme A concentrations in vitamin B<sub>12</sub> deficient animals. The rôle of acid phosphatase in the adrenals is not clearly known.

Kar (1950, 1951) suggests that testicular alkaline phosphatase is in some way related to testicular function. Kellerman (1955) has indicated the relationship between hexokinase and phosphatase in guineapig testes. The observed increase in the testicular alkaline phosphatase in our studies might be due to a changed physiological function of the organ.

The rôle of phosphatase in spleen, thyroid, and pituitary is not clearly known and hence the changes observed can not be explained from the results at hand. It only indicates a changed physiological function of the above organs. Concerning the hormonal regulation of enzymes, it is supposed that the enzymes are functioning under extremely complicated situations. Brachet and Jeneer (1948) and Bourne (1943) have suggested a rôle of alkaline phosphatase in nucleic acid metabolism which will be considered later. In view of the evidences at hand it would seem too premature to expound any theory on the rôle of phosphatases from the results of histochemical technics only.

The ribose and desoxyribose nucleic acids are found to be decreased in all the tissues except thyroid, pituitary, and kidney. The rôle of vitamin B<sub>12</sub> in

nucleoprotein metabolism is quite evident from the reports available. Sebrell and Harris (1954) have cited references to show that vitamin B<sub>12</sub> has an important rôle in nucleic acid synthesis, and consequently, in the deficient animals the nucleic acids are markedly diminished. Taking into account the reports of Brachet and Jeneer (1948) and Bourne (1943), the increased alkaline phosphatase may as well explain the diminution in nucleic acid synthesis, though the exact mechanism of action of phosphatase in nucleic acid synthesis is not clearly known. Our work regarding the nucleic acid content of tissues is in agreement with the works reported by Schweigert *et al.* (1954), Rasch *et al.* (1955), Rose and Schweigert (1952) and Wang *et al.* (1954).

Histological changes in the tissues of vitamin B<sub>12</sub> deficient animals have been observed by various workers. They are much more pronounced in the second generation as reported by Ferguson and Couch (1954). The degeneration observed by us in the testes may explain the gross degenerative changes and undergrowth in the second generation.

The changes observed in the thyroid might be due to the presence of iodinated casein in the diet.

The diminution of cytoplasmic basophilia in livers of rats deficient in vitamin B<sub>12</sub> and folic acid, confirms the reports of Stern, Taylor and Russell (1949). A heavy haemorrhage and fat infiltration to some extent were also observed which may be due to the depletion of the vitamin B<sub>12</sub> stores in the body, which is a lipotropic agent. The spleen shows a considerable increase in the number of nuclei in deficiency, which seems to account for the increase in the DNA content of the deficient spleen.

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Mrs. Maya Mukerjee kindly carried out the haematological studies and microbiological assay of vitamin B<sub>12</sub> and folic acid contents of the liver to establish deficiency in the rats with respect to the two vitamins.

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# NEW PLANT RECORDS FOR THE UPPER GANGETIC PLAIN

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## ABSTRACT

Although great advancement in researches in practically all other branches of botany has been made in India during recent years, floristic and vegetational studies have been greatly neglected since the publication of Hooker's Flora of British India and other regional floras in the latter part of nineteenth and early twentieth century. During the last two decades or so fortunately, some interest has again been revived in this subject and attempts have been made to study local vegetation of various parts of this great sub-continent. Concerted action on the part of foresters and botanists in various universities and other scientific organisations and research institutions is, however, necessary in the collection of fresh and adequate material for floristic studies and thereby help in the revision of the Flora of India.

The present paper is the fifth of the series dealing with the author's studies of the Flora of the Upper Gangetic Plain and the adjacent Siwalik and sub-Himalayan tracts, which cover an area of about 1,96,000 sq. miles (5,07,640 sq. Km.). Most of the materials on which this paper is based have been collected or scrutinized by the author within recent years and are deposited in the Dehra Dun Herbarium.

Modern nomenclature has been used throughout and 28 species referable to 20 genera and 10 families, hitherto not reported from the area by previous workers, have been listed. One new combination in the family *Convolvulaceae*, viz., *Argyria bella* (Clarke) Raizada has been made. For the benefit of Indian workers detailed descriptions with critical notes of species not described in Hooker's Flora of British India have been given.

Floristic and vegetational studies have generally been greatly neglected in India since the publication of the monumental 'Flora of British India' by Sir J. D. Hooker and other provincial floras in the latter part of the nineteenth and early twentieth century. During the last decade or so, fortunately interest has again been revived to a certain extent in this subject and attempts have been made to study the local vegetation of various parts of the country (J. Banerji, 1948 ; M. L. Banerji, 1952*a, b*, 1953 ; Chatterjee and Bharadwaja, 1955 ; Ghildyal, 1957 ; Govindu, 1949 ; Govindu and Thirmulachar, 1952 ; Gupta, 1956 ; Jain and Bharadwaja, 1949 ; Joshi, 1956 ; Kingdon-Ward, 1948, 1949 ; Krishnaswamy, 1952 ; Mooney, 1947, 1950 ; Mudaliar and Kamath, 1954 ; Mukerjee, Sushil, 1947, 1953, 1956 ; Mukherjee, Sunil, 1953 ; Nair and Nathawat, 1956, 1957 ; Nasir, 1957 ; Navalkar, 1956 ; Patil, 1956 ; Patnaik, 1956 ; Patnaik and Patnaik, 1956 ; Raizada, 1948*a, b*, 1949, 1951, 1952, 1954*a, b, c*, 1957 ; Sahni, 1953 ; Sahni and Raizada, 1955 ; Santapau, 1947, 1950, 1951, 1953*a, b, c, d*, 1955 ; Santapau and Raizada, 1956 ; Seshagiri Rao, 1953 ; Shanti Swarup, 1957 ; Srivastava, 1955*a, b*, 1956*a, b* ; Stewart, 1951 ; Thirumalachar, *et al.*, 1949 ; Watts, 1954 ; etc.). This will doubtless greatly help in the revision of the local floras and the flora of India, provided such interest is kept up by the various Indian universities and other scientific and research organisations and institutions and proper facilities are made available for this purpose.

The present paper is the fifth of the series dealing with the author's studies of the 'Flora of Upper Gangetic Plain and the adjacent Siwalik and sub-Himalayan Tracts'.\* Most of the materials on which this paper is based have been collected

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\* Recently introduced or otherwise imperfectly known plants from the Upper Gangetic Plain. Pt. I. *Journ. Ind. Bot. Sci.*, 14, 339-348, 1935. Pt. II. *Ibid.*, 15, 149-167, 1946. Pt. III. *Ind. For. Rec. (N.S.) Botany*, 1, 223-235, 1939. Pt. IV. *Ibid.*, 4, 65-72, 1950.

or scrutinized by the author within recent years and is lodged in the Dehra Dun Herbarium.

In addition to the species not previously described by Duthie in his flora or noted and recorded by the author as occurring in the Upper Gangetic Plain, Sunil Mukherjee mentions the following species as having been collected on the Delhi Ridge : *Atylosia elongata*, *Blepharis asperima*, *Leucas diffusa* and *Opuntia dillenii*. In addition Srivastava (1956) mentions the species listed below as having been collected by him, but so far these have not been reported from any part of Uttar Pradesh : *Athroisma laciniatum*, *Bulbostylis capillaris*, *Convolvulus microphyllus*, *Luffa graveolens* and *Vicoa cernua*.

The woody flora of the area under consideration is now practically completely known but it is probable that some species, particularly of herbaceous and weedy plants, still remain to be collected and that, future intensive and careful botanizing in the region will add a few more to the present list. Furthermore, a number of species, especially of recently introduced exotics, which are commonly cultivated in gardens have begun to run wild and will doubtlessly become sufficiently common to deserve a place in the flora as much as the indigenous species.

As in previous parts the sequence of families and genera is that adopted by Sir J. D. Hooker in the Flora of British India and with few exceptions the families are enumerated without change in nomenclature and terminology. The generic and specific names have, however, been amended in accordance with the latest International Code of Botanical Nomenclature, but where changes have occurred the synonym as it appears in the Flora of British India is given. For the benefit of Indian workers detailed descriptions of those plants which do not find a place in Hooker's flora and of which it may, therefore, be difficult or inconvenient to find descriptions, are also added.

It has been found necessary to make one new combination, *Argyreia bella* (Clarke) in the family *Convolvulaceae*.

#### CRUCIFERAE

*Lepidium ruderae* Linn. Sp. Pl. 645, 1753 ; Hk. f. Fl. Br. Ind. 1 : 160, 1872.

'New Forest, Dehra Dun, 15-5-1953, M. B. Raizada Dehra Dun Herb. No. 113072 and 113073 ! An abundant weed in waste land'.

An annual foetid herb with slender tap-root and a single, more or less erect, stem 10-30 cm. high. Flowers inconspicuous, greenish-white. Pods (siliculae), 2-2.5 mm. by 1.5-2 mm., retuse or notched at the tip.

This is a temperate Himalayan herb which is common in the hills, 2,000-4,000 m. Its occurrence in Dehra Dun is presumably due to its seeds having been brought down in the streams.

*Lepidium perfoliatum* Linn. Sp. Pl. 643, 1753.

'Laxmi Road, Dalanwala, Dehra Dun, 20-2-1953, M. B. Raizada Dehra Dun Herb. No. 112790 ! A herb near water channel, not common'.

An annual (or biennial ?) herb with single erect stem 20-40 cm., sparsely hairy, usually branched above. Basal leaves upto 10 cm., long-stalked, bipinnate, the ultimate segments less than 1 mm. wide ; middle and upper stem leaves 1-1.5 by 1-1.5 cm., broadly ovate, or deeply heart-shaped, acute, entire, clasping the stem with large basal lobes, strikingly different from the basal leaves. Flowers small, inconspicuous, in dense terminal ebracteate racemes. Petals pale-yellow, half as long as the sepals. Stamens 6. Fruiting-stalks ascending, glabrous, almost equalling the fruit ; siliculae variable in shape but usually about as broad as long, 3-4 mm.; valves keeled below, very narrowly winged above ; style usually projecting beyond the apical notch of the fruit. Seeds 4-6 in a cell, pale, smooth.



This species is a native of E. Europe and W. Asia and is now introduced throughout the rest of Europe and in N. Africa and N. America. It is not mentioned in the Flora of British India, although it has been collected from Baluchistan (Stocks 1008 in Herb. Dehra Dun) and Afghanistan (J. E. T. Aitchison 284 and S. A. Akhtar both in Herb. Dehra Dun). Its occurrence in Dehra Dun is, therefore, a mystery to me, although I have collected and observed it on several occasions. In all probability it has been introduced within recent times.

## FRANKENIACEAE

*Frankenia pulverulenta* Linn. Sp. Pl. 332, 1753; Hk.f. Fl. Br. Ind. 1 : 212, 1874.

'Sahibabad Daulatpur, Delhi, Feb. 1950, M. B. Raizada Dehra Dun Herb. No. 113649! A small spreading herb with deposit of salt crystals on all parts of the plant'.

'Delhi, Feb. 1951, Harbhajan Singh Dehra Dun Herb. No. 115559!'

A slender, exceedingly branched, diffuse herb with articulate branches and small opposite leaves. Flowers inconspicuous, small, solitary in the forks of the branches.

It is common in Sind and the plains of the Punjab. Although not recorded by Kashyap and Joshi in their 'Flora of Lahore district' it was collected by Dr. J. L. Stewart from Lahore as early as April 1864 (Stewart 2900 in Herb. Dehra Dun).

## CAESALPINIACEAE

*Caesalpinia digyna* Rottler in Ges. Naturf. Freunde Neue. Schr. 4 : 200, 1803; Hk. f. Fl. Br. Ind. 2 : 256, 1878.

'Domakhand, Gorakhpur, 25-2-1918, Sri Ram 937! A large climber, rare'.

A large scandent shrub, armed with hooked prickles. Flowers in racemes, pale-yellow. Pods shortly stalked, glabrous, fleshy, oblong, with a short recurved beak, 1-4 seeded, rich in tannin.

## COMPOSITEAE

*Artemisia parviflora* Buch. Ham. ex Roxb. Hort. Beng. 61, 1814; Hk. f. Fl. Br. Ind. 3 : 322, 1881.

'Dehra Dun, 12th Sept., 1870, Dr. King', in Herb. Dehra Dun.

'New Forest, Dehra Dun, 6-10-1955, M. B. Raizada Dehra Dun Herb. No. 123198. A tall herb with greenish-white flower heads in racemes.'

'Soil Conservation Farm, Bainkhala, Dehra Dun, Sept. 1957, S. S. Mehta, Dehra Dun Herb. No. 1123200.'

An erect, more or less aromatic, shrub-like herb. Lower leaves sessile, wedge-shaped or obovate-oblong, with generally a pair of stipule-like narrow lobes at the base.

This temperate Himalayan species which usually occurs between 1,500-3,500 m., is common in hilly districts throughout India. Its occurrence in Dehra Dun is apparently due to its seeds having been washed down by the streams.

## CONVOLVULACEAE

*Argyreia bella* (Clarke) Raizada com. nov.; *Lettsomia bella* Clarke in Hk. f. Fl. Br. Ind. 4 : 192, 1883.

'Songarh, Nepal border of Gonda, 1-1-1922, Sis Ram (Kanjilal's collector), Dehra Dun Herb. No. 116419!'

A pretty climber with milky juice and white tomentose branches. Leaves ovate-cordate, large; softly hairy above, white tomentose beneath. Flowers pink, campanulate. Fruit scarlet, dry when ripe.

## SCROPHULARIACEAE

*Alectra thomsoni* Hk. f. in Fl. Br. Ind. 4 : 297, 1884.

'Banda, U.P., Nov., 1921, Sri Ram Dehra Dun Herb., No. 90375. An erect leafless herb, flowers yellow. Reputed to be medicinal and a potent tonic. Local name 'Nirgundi, apparently parasitic'.

An erect almost leafless herb, often with several erect branches from near the base. Leaves scale-like ; flowers in racemes, yellow.

## ACANTHACEAE

*Staurogyne polybotrya* (Nees) O. Kuntze Rev. Gen. 1 : 407, 1891.; *Ebermaiera polybotrya* Nees ; HK. f. Fl. Brit. Ind. 4 : 396, 1884.

'Gorakhpur, Feb., 1956, S. K. Seth, Dehra Dun Herb. No. 113553. A herb'.

A small diffuse herb with opposite leaves and small purplish flowers, mostly in spikes, terminating the stems or on axillary branchlets almost bare of leaves at the base.

## POLYGONACEAE

*Polygonum chinense* Linn. Sp. Pl. 363, 1753 var. *ovalifolia* Meissn. in DC. Prod. 14 : 130, 1856 ; Hk. f. Fl. Br. Ind. 5 : 45, 1886.

'New Forest, Dehra Dun, 6-1-1955, M. B. Raizada Dehra Dun Herb. No. 123199. An undershrub about 3 ft. high and with white flowers. It has now escaped from cultivation and is running wild in our plantations'.

An erect or rambling shrub with ovate, or ovate-oblong leaves which are often subcordate at the base and white flowers.

This shrub which is frequently grown in gardens in Dehra Dun has now started to run wild and will very soon deserve a place in our flora like other indigenous species.

## LILIACEAE

*Lilium wallichianum* Schultes f. Syst. Pl. 7 : 1689 ; Hk. f. Fl. Br. Ind. 6 : 349, 1892.

'Songarh, Tulsipur Range, Gonda, 19-9-1921, Sis Ram Dehra Dun Herb. No. 118192 ! A herb'.

A perennial herb about 1-2 m. high and with large, narrowly linear leaves. Flowers subsolitary, large, funnel-shaped, white, sweet-scented.

## CYPERACEAE

*Cyperus atkinsoni* C. B. Clarke in Journ. Linn. Soc. 21 : 109, 1884 ; Hk. f. Fl. Br. Ind. 6 : 603, 1893.

'Delhi, 20-8-1952, M. B. Raizada Dehra Dun Herb No. 113198 !'

A perennial, rhizome creeping, stem 5-20 cm. long, trigonous below, terete above. Umbels simple often contracted into a head.

*Cyperus alulatus* Kern in Reinwardtia 1 : 463, 1952 ; *Cyperus iria* var. *rectangularis* Kükenth. in Engl. Pflanzenr., Heft 101 : 152, 1935 ; *Cyperus iria* ((non-Linn.) *Sensu* Clarke. Cyper. pl. 14 f. 1 : 1909, non al.

'Moradabad, Aug. 1843, Thomson 280' !

'Nalapani road, 2000 ft., Oct. 1891, J. S. Gamble 23192'.

'Dehra Dun, 2000 ft. Aug. 1891, Gamble 23852'.

'Gwalior, C. Maries 356'.

Annual. Stems erect, slender, triangular, smooth, 1-7 cm. long, 1-2 mm. broad, few foliate below. Leaves shorter than or as long as the stem, entire, soft,

long-acuminate, scabrous on the upper surface, 1.5 mm. broad. Inflorescence simple or somewhat compound, lax. Bracts obliquely patent, 2-4, elongated, similar to leaves, very prominent. Inflorescence (umbel) 3-9 radiate, bracteoles tubular, somewhat obliquely truncate, posteriorly cuspidate or bidentate, base brownish, 0.5-1.5 cm. long, emarginate, unequal, obliquely patent, slender, compressed, smooth, or with hirsute apex; umbels up to 16 cm. long sometimes longer, apex few-branched, rays short, pale-yellow with a tail like support (appendage). Spikes ovate or oblong-ovate, lax or somewhat dense, 1-3 cm. long, 8-25 (30) mm. broad with 5-20 spikelets; rachis flexuose, angular, somewhat hairy. Spikelets compressed, rectangularly divaricate or somewhat reflexed, ovate to oblong-linear, 3-12 mm. long, 2-2.5 mm. broad, 4-18-flowered, base enclosed and supported by a subulate seta. Rachilla obscurely brown, straight, somewhat wingless, internodes 0.6-1 mm. long. Glumes membranaceous,  $\frac{1}{3}$ - $\frac{1}{2}$  partly imbricate, somewhat spreading, concave, almost orbicular, (1.75)-2 mm. long and broad, emarginate below the apex, mucronulate, dorsal surface with 7 greenish nerves with one orange-yellow purplish nerve on one side; keel acute, bow-shaped, narrowed on the upper surface, wings spinulose-ciliate. Stamens 2, anthers small, oblong, sometimes linear, connective without an appendage and slightly prolonged. Style almost absent, stigmas 3; fruits many, small. Fruit circular in outline, obovate, three-angled, laterally concave, base broadly stipitate, apex mucronate, dark-brown, smooth and shining, densely pectinate, 1.5 mm. long, 0.8-0.9 mm. broad.

This species is close to *C. iria* L. which differs in glabrous rachis. Spikelets are 1.5-2 mm. broad, erect and then become spreading; small glumes are 1.25-1.5 mm. long. It is dorsally 3-5 nerved with keel wingless and smooth; and fruits small being 1-1.25 mm. long.

#### GRAMINEAE

*Setaria megaphylla* (Steud.) Dur. et Schinz. Consp. Fl. Africa 5: 773, 1895; Bo<sup>t</sup> in Kew Bull. 550, 1954; *Panicum megaphyllum* Steud. Syn. Pl. Glum. 530, 1854.

'Dehra Dun, Oct. 1890, Duthie 10755 and 10756'.

'Forest School Garden, Dehra Dun, Oct. 1892, Gamble'.

A perennial grass from a stout rhizome. Culms 1-3.5 m. tall, very robust, erect from the base, simple or branched, terete, smooth and glabrous or rather rough below the inflorescence. Leaf-blades elliptic-linear, tapering to the base, drawn out gradually to a long acuminate tip, glabrous or loosely hairy on the upper surface, coarsely and extremely scabrid on the margins and on the outer nerves on the upper surface, scabrid but less so on the under surface and in the centre on the upper surface, rigid, flat, pleated and somewhat crinkled towards the base; sheaths terete, striate, tightly clasping, glabrous and smooth below, pilose towards the collar and densely ciliate on both margins with tubercle-based hairs; ligule a dense fringe of hairs.

Inflorescence a linear to linear-lanceolate panicle, dense or loose, up to 30 cm. long by 10 cm. wide; axis angled, striate, very scabrid to shortly hirsute on the angles, carrying branches which are single or more often in groups or false whorls; branches similar to the axis up to 6 cm. long, branching or rebranching, the uppermost gradually shorter. Spikelets solitary on the ultimate branchlets, seated on scabrid pedicels, the lateral sometimes without a supporting bristle, the terminal always with one, about 3 mm. long. Lower glume broadly ovate to rotundate, membranous, smooth and glabrous about 1-1.5 mm. long, 3-nerved; upper glume broadly ovate-oblong, 2-2.4 mm. long, 5-nerved. Lower floret empty; lemma membranous, elliptic-acute or apiculate, smooth and glabrous, 5-nerved; palea a short hyaline membrane. Upper floret hermaphrodite; lemma elliptic-apiculate, chartaceous to crustaceous, smooth or very obscurely rugulose, turning coffee brown

at maturity ; palea of the same texture ; stamens 3 ; anthers 1.25 mm. ; styles 2, distinct ; stigmas plumose, as long as the styles.

A native of Africa, introduced in India. Probably an escape within the area, but not yet naturalized.

*Arundinella setosa* Trin. Gram. Panic. 63, 1826 var. *setosa* Bor in Kew Bull. 391, 1955 ; *Arundinella capillaris* Hk. f. Fl. Br. Ind. 7 : 74, 1896.

'Mohan Pass, Saharanpur Siwaliks, Oct. 1898, Duthie'.

A perennial grass up to 1 m. tall with a densely tufted hard rootstock but without a rhizome ; base glabrous. Inflorescence a drooping panicle which is extremely variable in size and density. Spikelets 6.5–7.5 mm. long.

*Leersia hexandra* Sw. Prod. Veg. Ind. Occ. 21, 1788 ; Hk. f. Fl. Br. Ind. 7 : 94, 1896.

'Saharanpur, 20th August, 1851, Jameson'.

'Mala, Pilibhit district, 1-2-1918, Sri Ram Dehra Dun Herb. No. 60088' !

'Gorakhpur, Nichaut road side, near paddy fields, 5-11-1950, M. B. Roizada 133/1950' !

An aquatic perennial grass, common in swamps. It is said to provide good fodder.

*Aristida depressa* Retz. Obs. 4 : 22, 1786 ; *A. adscensionis* Linn. Sp. Pl. 82, 1753 ; Hk. f. Fl. Br. Ind. 7 : 224, 1896 in part.

'Ajmer, 1883, Lowrie 4935'.

'Baghpat, Meerut district, 11-12-1885, Duthie 4934'

'Etawah, 26-11-1886, Duthie 6574'

'Aligarh, 7-11-1887, Duthie 6771'

A xerophytic grass of arid and semi-arid regions, preferring dry and sandy localities. It is common throughout the area.

This species differs from *A. adscensionis* Linn. mainly in the very unequal length of the glumes, the lower glume is about  $\frac{2}{3}$  as long as the upper and both are moreover very acute, the lower distinctly being awned, while the upper is without a bifid apex and is slightly pointed.

*Alopecurus geniculatus* Linn. Sp. Pl. 60, 1753 ; Hk. f. Fl. Br. Ind. 7 : 239, 1896.

'Dehra Dun, Umrao Singh 316'

An annual or semi-perennial grass ; culms erect or geniculate at the base, rooting at the nodes, 20–60 cm. tall, stout or slender. Inflorescence a cylindric or oblong, spiciform panicle 2.5–7.5 cm. long. Spikelets strongly compressed, 2.75 mm. long.

*Garnotia elata* (Arn. ex Miq.) Janowski in Fedde, Rep. Spec. Nov. 17 : 86, 1921 ; *Berghausia elata* Arn. ex Miq. Analect. Bot. 2 : 20 ; *Garnotia scoparia* Stapf ex Hk. f. in Fl. Br. Ind. 7 : 242, 1896.

Gomti Bandha, Lucknow, Nanheram Dehra Dun Herb. No. 42695. A tall grass growing in tufts of culms'.

A tall grass up to 1 m. high ; leaves straight rigid with scabrid margins ; sheaths with woolly margins. Panicles long upto 1 m., very narrow.

This grass is so far known to occur in South India (Madras State) only. Its find in Lucknow is therefore inexplicable and a puzzle to the author as it has not been reported to occur in between.

\**Sporobolus stocksii* Bor in Kew Bull. 45, 1948 ; *Sporobolus ioclados* Hk. f. Fl. Br. Ind. 7 : 249, 1896, non Nees Fl. Afr. Austral. 161.

'Merwara, 1884, A. E. Lowrie 5239'

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\*While this paper was going through the press I got an opportunity to examine a fragment of the type (J. E. Stocks) of *Sporobolus stocksii* Bor, supplied to me through the courtesy of the Director, Royal Botanic Gardens, Kew. This revealed that Lowrie's specimen is not *S. Stocksii*. Consequently it was sent to Dr. Bor of Kew for his opinion who just informs me in his letter dated 17.4.58 that "it is not *S. Stocksii* but I have not matched it well ; it is like *S. minutiflorus* with longer spikelets and may prove to be a distinct species."

A densely tufted rather stout grass with densely tufted narrow leaves. Panicle branches flexuous, opposite and alternate; upper lemma constantly shorter than the lemma. In the true *Sporobolus ioclados* Nees which is a native of South Africa, the panicle branches are straight and verticillate and the upper glume is equal to or slightly longer than the lemma.

*Sporobolus helvolus* (Trin.) Th. Dur & Schinz. Consp. Fl. Afric. 5 : 820, 1895; *Vulfia helvola* Trin. in Mem. Acad. Petersb. Ser. VI Se. Nat. v. II. 52, 1840; *Sporobolus glaucifolius* Hk. f. Fl. Br. Ind. 7 : 247, 1896 non Hochst.

'Ajmer, B. Tiagi, Dehra Dun Herb No. 15/101560'

A perennial grass.

*Sporobolus violascens* Mez.\* in Fedde, Rep. Sp. Nov. 17 : 298, 1921.

'Gwalior', Fide Mez.

Apparently an annual, about half metre long, slender. Leaf-sheath much shorter than the culm internode, not at all keeled, with glabrous margins; ligule hairy, short, variable, not barbate on the sides; lamina totally longitudinally convolute on drying up, margins thickened a little and somewhat serrate near the base, not conspicuously ciliate. Inflorescence, more or less, many flowered, lax, tri-pinnately paniculate, sub-pyramidal in shape, about 0.14 m. long and 55 mm. broad; all branches remarkably verticillate, about  $\frac{1}{3}$  or  $\frac{1}{2}$  arrow pointed in shape. Spikelets very long-pedicelled, about 2 mm. long. Glume I elliptic-rotundate, without nerves, scarcely more than  $\frac{1}{5}$  the length of the spikelet; glume II nearly as long as the spikelet, broadly rotundate, thinly 1-nerved. Lower palea (lemma) as long as the spikelet, apex obtusely denticulate, 1-nerved; upper lemma (palea proper) much above and a little smaller than the lemma, laterally truncate, apex denticulate as in the lemma.

*Eragrostis tremula* Hochst. ex Steud. Syn Gram 269, 1854; Hk. f. Fl. Br. Ind. 7 : 323, 1896.

'Shahjahanpur, 10-10-1885, Duthie 5111'

'Ajmer, Lowrie'

'Etawah, 26-11-1886, Duthie 6597'

'Mailani, South Kheri, 12-11-1920, Sri Ram'

'Banda, 22-10-1921, Sri Ram'

'Jaipur, 2-2-1957, Raizada's collector 25392'

This grass is fairly common throughout the Upper Gangetic Plain, Behar, Bengal, Assam, Kathiawar and the Western Ghats; also in Afghanistan and tropical Africa.

It prefers light soils and is said to be a good fodder, but the foliage yield is small.

*Eragrostis poaeoides* Beauv. Ess. Agrost. 162, 1812; Hubbard in Kew Bull. 17, 1933; *E. minor* Host. Gram. Austr. 4 : 15, 1809 (in nota) et in Fl. Austr. 1 : 135, 1827; Stapf in Hk. f. Fl. Br. Ind. 7 : 321, 1896.

'Saharanpur, Royle 109'

'Dehra Dun, July 1894, Gamble 24664'

'Gonda, 20-5-1918, Sri Ram'

'Ajmer, Lawrie 4958'

It is an annual grass common in fields during the rains and winter throughout the area.

The name *Eragrostis minor* Host. is untenable since the genus was not validly published until 1812.

\* Mez., l.c., mentions that the type is in the Calcutta Herbarium. In spite of prolonged search by me and by the Keeper, Dr. S. K. Mukerjee, the type, however, could not be traced. Dr. N. L. Bor, Assistant Director, Royal Botanic Gardens, Kew to whom I referred this matter just informs me in his letter dated 13-3-58 that "about 8 years ago I wrote to Biswas for *Sporobolus violascens* Mez and it could not be traced in the Calcutta herbarium. I have tried to run it down in Berlin and in other herbaria in Germany also without success".

In absence of the type, although it is rather difficult to comment on the status of this species which, no doubt, is distinct, it appears to me from its description that this may be the same as *Sporobolus tetragonus* Bor, subsequently described in the Kew Bull. 1949, p. 251.

*Poa infirma* H. B. K. Nov. Gen. et Sp. 1 : 158 (1815) 27 ; Bor in Journ. Bom. Nat. Hist. Soc. 50 : 818, 1952 ; *Poa annua* Linn. ssp. *exilis* Tomm. apud Freyn. Zool. Bot. Ges. 27 : 469, 1877 ; *Catabrosa thomsoni* Stapf ex Hk. f. Fl. Br. Ind. 7 : 311, 1896.

'Robber's Cave, 780 m., 29th Feb. 1928, Umrao Singh 317'

A strictly annual grass. Culms rather slender and weak, smooth and glabrous, up to 10 cm. tall, occasionally twice as tall, sheathed almost to the inflorescence. Leaf-blades soft, flaccid, linear, abruptly contracted to a blunt point, up to 6 cm. long, 5 mm. broad, scabrid on the margins and on the midrib below, very scabrid at the tip, very thin. Sheaths rather loose, herbaceous, smooth and glabrous, somewhat inflated at the base of the plant. Ligule membranous, entire, 1-2 mm. long, rounded or obtuse at the tip.

Inflorescence a narrow, oblong, rather open panicle with branches ascending, rarely horizontal, and never deflexed ; axis smooth and glabrous, angled ; branches smooth and glabrous, in pairs, often a longer accompanied by a shorter, up to 2 cm. long, carrying rather remote spikelets at anthesis. Spikelets 4-4.5 mm. long, 3-5 flowered, oblong-obtuse in shape, with remote florets which occasionally hide the joints of the rachilla, seated, except the terminal, on very short pedicels. Lower glume 1.25 mm. long, 0.6 mm. wide, oblong-acute in shape, slightly curved on the back, broadly hyaline on the margins, smooth and glabrous. Upper glume 1.5 mm. long, 1 mm. wide, broadly elliptic-obtuse in shape when flattened, very broadly hyaline on the margins and at the tip, 3-nerved, smooth and glabrous. Lemma 2.5 mm. long, 1.5 mm. wide, widest above the middle, oblong-ovate-obtuse or almost round at the tip, herbaceous in texture, faintly 5-nerved, very broadly hyaline at the tip and along the margins, almost straight on the back thickly ciliate on all nerves or occasionally thinly ciliate. Wool absent. Rachilla produced and carrying a rudimentary spikelet, smooth and glabrous. Anthers minute, 0.22-0.33 mm. long. Palea shorter than the lemma, long ciliate on the keels.

This delicate little species is comparatively rare and is strictly annual. It bears only a superficial resemblance to *Poa annua*. The panicle is oblong in shape. All lemmal nerves are hairy, but there is no wool at the base of lemma. The anthers are hermaphrodite. The leaves are remarkably thin and are almost translucent.

*Aeluropus lagopoides* (L.) Druce in Rep. Bot. Ex. Club. Br. Is. 15 : 603, 1917 ; *Dactylis lagopoides* Linn. Mant. 33, 1767 ; *Aeluropus villosus* Trin. ex C. A. Mey-Verz. Pfl. Cauc. 18, 1831 ; Hk. f. Fl. Br. Ind. 7 : 334, 1896.

'Model Town, Delhi, August 1953, M. B. Raizada 129/1953, growing on sandy bank of a stagnant water canal'.

A low much branched perennial grass. Distributed throughout the Punjab, Sind and Western Peninsula in salt ground, also on alkaline soil.

*Vulpia megalura* (Nutt.) Rydb. in Bull. Torrey Bot. Club. 36 : 538, 1909 ; Bor in Journ. Bom. Nat. Hist. Soc. 50 : 342, 1951 ; *Festuca megalura* Nutt. in Jour. Acad. Philad. n.s. 1 : 188, 1848.

'Saharanpur, March 1891, G. Wingate'

An annual grass. Culms up to 60 cm. tall, slender to somewhat robust, smooth and glabrous, leafy almost to the panicle, striatulate, terete, glabrous on the nodes. Leaf-blades linear, long acuminate, soft to rather stiff, flat or plicate, rolled or involute, up to 20 cm. long, 1.5-3 mm. wide, puberulous on the upper surface with short, soft, white hairs, glabrous on the lower surface, scabrid along the nerves on the upper surface and also on the margins, smooth on the lower surface ; leaf-sheaths tight or loose, the upper somewhat inflated and containing the inflorescence, markedly striate, smooth and glabrous with hyaline margins which are continuous with the ligule, often longer than the internodes ; ligule a hyaline membrane, 0.5-1 mm. long.

Inflorescence a strict, narrow panicle, nodding or erect, with short appressed branches, bearing few spikelets which are secund, 6-25 cm. long, at the most 2 cm. broad; rhachis triangular in cross section, winged on the angles, scabrid on the wings, pale with greenish wings, glabrous, branched; branches short, angled and scabrid on the angles, inflated above just below the spikelet, fascicled, binate or solitary. Spikelets about 15 mm. long, without the awns, 3-6-flowered, secund. Lower glume 2-2.5 mm. long, subulate, acicular, 1-nerved, hyaline on the margins, smooth or glabrous or minutely scabrid, nerve green. Upper glume 3.5-5.5 mm. long, acicular, 1-nerved, subulate in outline, setaceously acuminate, smooth and glabrous, or slightly scabrid on the dorsal surface towards the tip. Lemma 6.5-7.5 mm. long, narrowly elliptic-acute, 5-nerved, the central nerve passing out into a scabrid awn 10-20 mm. long or more, coarsely scabrid on the dorsal surface especially towards the tip, furnished with white hairs on the upper half of the margins of the upper lemmas (hairs often missing from the lowest lemma); palea shorter, 2-keeled, coarsely scabrid on the keels; stamen I; anther 1 mm. long; mature caryopsis not seen.

This American species has been frequently confused with *Vulpia myuros* (Linn.) Gmel. (*Festuca myuros* Linn.) in various collections. The former however, differs from the latter in that the lower glume is almost 1.5 mm. long and the furnished lemmas are with long hairs on the margins while in *Vulpia myuros* the lower glume is 2.5-3 mm. long and the lemmas are hyaline on the margins.

Apparently this grass, because of its fodder value, was cultivated at Saharanpur, along with others and has really not acclimatised or naturalized in our area.

N.B. While this paper was in the press I got further opportunity to critically examine the material of the genus *Tripogon* lodged in the Dehra Dun Herbarium. In addition to *T. lisboae* Stapf already recorded from the Upper Gangetic Plain, *T. filiformis* Nees ex Steud. (Dehra Dun, Duthie 6862, 7761 & 10774) and *T. roxburghianus* (Steud.) Bhide (Gwalior, C. Maries 77) are also found with in the area.

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# MORPHOLOGICAL CHARACTERS OF THE HUMAN FOOT

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## ABSTRACT

1. The contour method of studying the morphology of the human foot and its deviations from the International Agreement of Anthropometric Measurements have been described.

2. The human foot can be divided into three types on the basis of the relative lengths of the toes ; (i)  $1 > 2$  ; (ii)  $2 > 1$  and (iii)  $1 = 2$ . Following Minami they have been called T, F and O respectively. The different homo- and heterotypes have been described.

3. In both the rural and urban females the type F occurs in a frequency higher than the males and it appears to be sex-limited in nature.

4. A scheme of 11 types of interspaces between the toes has been proposed. The females, both rural and urban, show more interspaces than the males. The influence of the footwear on the interspaces has been discussed.

5. The forms of interspaces appears to be hereditary.

6. The anthropometric characters, measured from the foot contours of 7 Bengal castes and 5 aboriginal tribes of Bihar and Orissa, have been discussed.

## INTRODUCTION

Among the morphological characters of the human foot, the relative lengths of the first and the second toes have been the subject of some studies. The largest variability, however, appears to occur in the four interdigital spaces (*interstitium* of Martin) between the five toes, which have not been, as far as the present writer's knowledge goes, studied in detail. Martin (1928) appears to have emphasized this character of the foot, as seen in the interspace between the hallux and the second toe, and his contour drawing of the Senoi feet gives an excellent idea of all the interspaces between the toes.

A study of the *interstitium* of the toes is only possible from accurate contour tracing of the foot. The study of the contour tracings was recommended at the International Agreement of Anthropometric Measurements at Geneva in 1912 (Stewart, 1947) but so far, this method has practically received no serious study. The Geneva Agreement did not recommend any specific measurement on the contour tracings. Martin recommended this method of study and Osman Hill (1941) has applied it in his study of the Vedda foot. The contour method facilitates the study of the many morphological characters of the foot, *i.e.*, the length and breadth, the relative lengths of the first and the second toes, the angle of divergence of the hallux and the four *interstitia* between the toes. Thus, with these eight morphological characters it can serve an useful purpose of identification in criminology and forensic medicine.

In the present paper the contour method of study has been applied on five aboriginal tribes of Bihar and Orissa and a few caste groups, urban and rural, from Bengal. The urban Bengali data have been collected from the city of Calcutta and the rural data from the village of Dakshingram, Birbhum, West Bengal.

## METHOD OF STUDY

In taking the contour of the foot, the subject is seated on a low stool so that the foot reaches the ground. The subject is then asked to place his foot lightly on a sheet of paper, the leg being perpendicular to it. The investigator then kneels before the toes of the subject and the left hand is placed on the arch of the foot. A thin pencil, with the lead exposed long, is held vertically and the outline is started from the middle point of the heel. From this point the outline is traced along the lateral margins of the foot. The two lateral borders being thus drawn, the toes and the interspaces in between them are carefully drawn. Only the natural interspaces are drawn and the lead of the pencil is slipped in only when an interspace is visible before the meeting point of the two toes. Before the subject is asked to remove his foot the two landmarks, metatarsale tibiale and metatarsale fibulare, are marked on the outline.

The above method thus appears to be slightly different from that suggested by the International Agreement. The latter recommends the contour of the foot to be drawn in the same manner as the hand in which, the fingers have to be "very slightly separated". It appears to the present writer that this process is not probably possible in the toes, firstly, because of their much lesser lateral mobility and secondly, any attempt at movement will cause distortion of the contour of the whole foot. The toes are difficult to be separated singly and laterally, like the fingers. The other recommendation of marking "the extreme end of each interdigital cleft" with a dot is not probably possible in all feet. Its purpose is also not explained. The extreme end of the interdigital cleft, where it is easily visible, will automatically be drawn in the procedure followed in the present study ; but where the extreme end of the interdigital cleft is not visible, it has to be mechanically splayed out for dotting, which is likely again to distort the contour drawing. It is, therefore, desirable to look for the natural interspaces only.

The inner border of the foot is, of course, "always unreliable" but to complete a contour and to give the shape of a foot, it should be drawn and not left out.

## RELATIVE LENGTHS OF FIRST AND SECOND TOES

The hallux usually attains the greatest length while the second toe occasionally surpasses the first toe in length. The third toe is also found to be the greatest in length in very rare instances (Wood Jones, 1949). In some cases the hallux and the second toe are found to be of equal length. Thus, on the above basis there can be three types of human feet :

$$1 > 2 ; 2 > 1 ; 1 = 2$$

Hawkes (1912) proposed the symbols L (LL) for  $1 > 2$ , S (SS) for  $2 > 1$  and E (EE) for  $1 = 2$  for the main homotypes in the right and left feet while six other symbols were used for the six heterotypes (LS-A ; SL-B ; LE-C ; EL-D ; SE-G ; ES-H), the symbol on the left standing for the left foot and that on the right for the right foot. Minami (1952) proposed T (tibial) for the hallux being greater, F (fibular) for the second toe being greater while O for both the toes being equal. The last, according to him, is a transitional type. He did not propose any symbol for the heterotypes and remarked that "there are no clear differences in these frequencies of heterotypes". This appears to be true in the case of the data presented in this paper. The frequency of the homotypes is predominantly higher than those of the heterotypes. The foetal researches of Schultz (1924) and Minami show that there is a clear racial difference among the three races, White, Negro and Mongolian, in the frequency of each of the above types.

TABLE I  
*Relative Lengths of the I & II Toes (Rt & Lt)*

| Sr. No.       | Castes            | Sex    | No.      | 1 > 2<br>T   | 2 > 1<br>F  | 1 = 2<br>O | Remarks | Locality               |
|---------------|-------------------|--------|----------|--------------|-------------|------------|---------|------------------------|
| 1.            | Bengal Low Castes | male   | 160<br>% | 124<br>77.50 | 30<br>18.75 | 6<br>3.75  | Rural   | Birbhum<br>(W. Bengal) |
| 2.            | „ Artisan „       | „      | 134<br>% | 105<br>78.36 | 24<br>17.91 | 5<br>3.73  | „       | „                      |
| 3.            | „ Muslims         | „      | 54<br>%  | 40<br>74.07  | 12<br>22.22 | 2<br>3.70  | „       | „                      |
| 4.            | „ Brahmans        | „      | 130<br>% | 98<br>75.38  | 22<br>16.92 | 10<br>7.69 | „       | „                      |
| 5.            | „ „               | female | 52<br>%  | 36<br>69.23  | 15<br>28.85 | 1<br>1.92  | „       | „                      |
| 6.            | „ High Castes     | male   | 240<br>% | 214<br>89.17 | 18<br>7.50  | 8<br>3.33  | Urban   | Calcutta               |
| 7.            | „ „ „             | female | 100<br>% | 78<br>78.0   | 16<br>16.0  | 6<br>6.0   | „       | „                      |
| <i>Tribes</i> |                   |        |          |              |             |            |         |                        |
| 8.            | Juang             | male   | 86<br>%  | 79<br>91.86  | 3<br>3.49   | 4<br>4.65  | Rural   | Orissa                 |
| 9.            | Oraon             | „      | 88<br>%  | 82<br>93.18  | 5<br>5.68   | 1<br>1.14  | „       | Ranchi                 |
| 10.           | Pahira            | „      | 58<br>%  | 46<br>79.31  | 7<br>12.07  | 5<br>8.62  | „       | Manbhum                |
| 11.           | Mundari           | „      | 90<br>%  | 73<br>81.11  | 12<br>13.33 | 5<br>5.56  | „       | Ranchi                 |
| 12.           | „                 | female | 18<br>%  | 15<br>83.33  | 2<br>11.11  | 1<br>5.56  | „       | „                      |

Table I shows the frequency of the above three types in the various castes and tribes of Bengal, Bihar and Orissa. All of them predominate in having the high frequency of the hallux (type 1 > 2) being greater than the second toe and it is

TABLE II  
Frequency of the Homo & Hetero Types

| Sr. No.           | Castes            | Sex    | No.   | TT           | FF         | OO        | TF        | FT         | TO         | OT        | FO        | OF        | Remarks |
|-------------------|-------------------|--------|-------|--------------|------------|-----------|-----------|------------|------------|-----------|-----------|-----------|---------|
| 1.                | Bengal Low Castes | male   | 80 %  | 55<br>68.75  | 9<br>11.25 | —         | 8<br>10.0 | 2<br>2.50  | 3<br>3.75  | 1<br>1.25 | 1<br>1.25 | 1<br>1.25 | Rural   |
| 2.                | " Artisan "       | "      | 67 %  | 49<br>73.13  | 9<br>13.43 | —         | 2<br>2.99 | 2<br>2.99  | 1<br>1.49  | 2<br>2.99 | 1<br>1.49 | 1<br>1.49 | "       |
| 3.                | " Muslims         | "      | 27 %  | 18<br>66.67  | 3<br>11.11 | —         | 1<br>3.70 | 3<br>11.11 | —          | —         | —         | 2<br>7.41 | "       |
| 4.                | " Brahmans        | "      | 65 %  | 43<br>66.15  | 6<br>9.23  | 1<br>1.54 | 5<br>7.69 | 2<br>3.08  | 4<br>6.15  | 1<br>1.54 | 1<br>1.54 | 2<br>3.08 | "       |
| 5.                | " "               | female | 26 %  | 16<br>61.54  | 6<br>23.08 | —         | 1<br>3.85 | 2<br>7.69  | 1<br>3.85  | —         | —         | —         | "       |
| 6.                | " High Castes     | male   | 120 % | 104<br>86.67 | 6<br>5.00  | 2<br>1.67 | 1<br>0.83 | 3<br>2.50  | —          | 2<br>1.67 | 1<br>0.83 | 1<br>0.83 | Urban   |
| 7.                | " "               | female | 50 %  | 35<br>70.0   | 1<br>2.0   | —         | 5<br>10.0 | 3<br>6.0   | —          | 2<br>4.0  | 1<br>2.0  | 3<br>6.0  | "       |
| <i>Aboriginal</i> |                   |        |       |              |            |           |           |            |            |           |           |           |         |
| 8.                | Juang             | male   | 43 %  | 37<br>86.05  | —          | —         | —         | 2<br>1.72  | 3<br>2.58  | —         | 1<br>0.86 | —         | Rural   |
| 9.                | Orson             | "      | 44 %  | 39<br>88.64  | 1<br>2.27  | —         | 1<br>2.27 | 2<br>4.54  | 1<br>2.27  | —         | —         | —         | "       |
| 10.               | Pahira            | "      | 29 %  | 22<br>75.86  | 2<br>6.90  | 2<br>6.90 | 1<br>3.45 | 1<br>3.45  | —          | —         | 1<br>3.45 | —         | "       |
| 11.               | Mundari           | "      | 45 %  | 33<br>73.33  | 3<br>6.67  | 1<br>2.22 | 4<br>8.89 | 1<br>2.22  | 1<br>2.22  | 1<br>2.22 | —         | 1<br>2.22 | "       |
| 12.               | "                 | female | 9 %   | 7<br>77.78   | 1<br>11.11 | —         | —         | —          | 1<br>11.11 | —         | —         | —         | "       |

true in the case of the female groups as well. In the latter sex, however, excepting the Mundari females, the frequency of the type F ( $2 > 1$ ), appears in a much higher frequency than the males. Among the male rural Brahmans its frequency has been found to be 16.92 per cent in comparison to 28.85 per cent in the females. Among the urban high caste Bengali women its frequency of 16 per cent is more than double the male percentage of 7.5 per cent. The other type O ( $1 = 2$ ) occurs in a low frequency of 1.92 per cent among the rural Brahman females while it occurs in 7.69 per cent among the males. The urban samples, however, show just the contrary picture—the females (6 per cent) showing almost twice that of the males (3.33 per cent).

The rural and the urban samples of the two high caste groups also differ considerably from one another. Among the males the frequency of the type T shows a considerable increase among the urban peoples (89.17 per cent) as against 75.38 per cent of the rural group while the other two types F and O occur in much higher frequencies among the latter group than the former. Type F occurs in 16.92 per cent in the rural group as against 7.50 per cent in the urban group while the type O occurs in 7.69 per cent and 3.33 per cent in the rural and urban respectively. In respect of the female sex, the male order is retained in the case of types T and F while in the type O the urban females (6 per cent) show a higher frequency than the rural females (1.92 per cent).

It is difficult to explain this rural-urban difference at this stage though the use of shoes by the urban peoples deserves a mention. The second toe sometimes shows a curvature of the terminal phalanx and this might cause a reduction in its length when tight-fitting shoes are worn. Such a foot is likely to be diagnosed as belonging to type T.

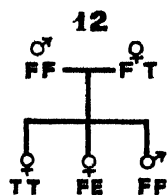
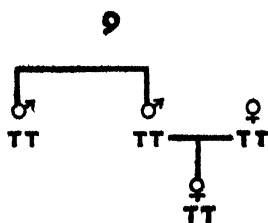
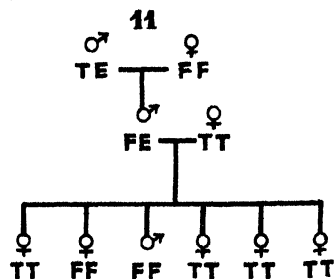
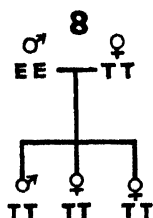
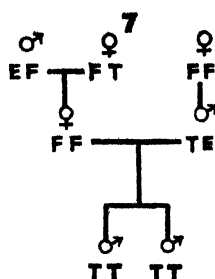
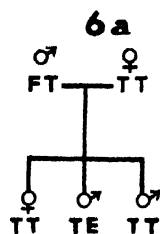
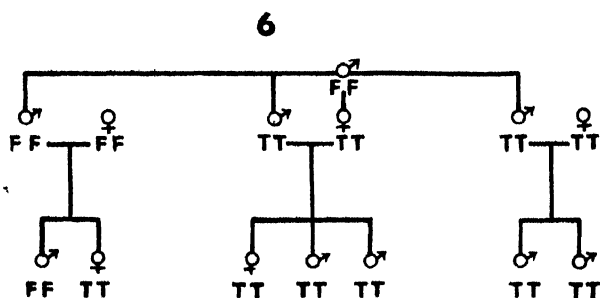
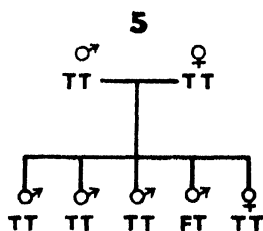
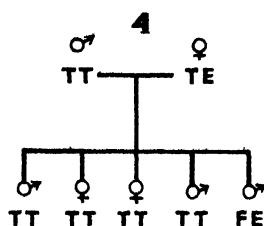
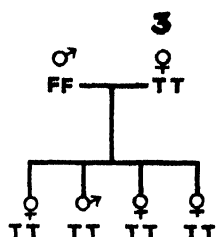
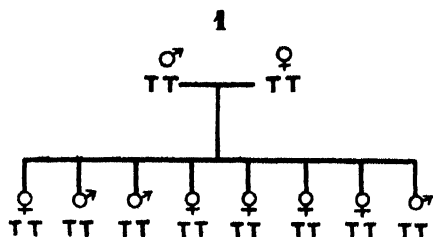
The sexual difference in the second toe appears to be genetic. Hawkes drew attention to the sex-limited nature of the F type of foot, which occurs more commonly in the females than in males. The T type of foot was also found to behave irregularly dominant over the F type of foot. According to Hawkes, besides the heterozygotes TF, FT, etc., both T (TT) and F (FF) behave as heterozygotes—the former in the males and the latter in the females.

In course of the present study, which for obvious reasons, has to be morphological first of all, some pedigrees were also collected and they will be discussed afterwards. Before we take up the pedigree material it will be worthwhile, first of all, to discuss the different homo and hetero-types in the general population. Hawkes has differentiated two heterozygotes, according to the right and the left limbs, *i.e.*, A for the right foot showing F and the left foot showing T, while B for the right foot showing L and the left foot showing S. In Table II the percentages of the various combinations have been shown :

It will be seen from the above table that the homotype TT occurs in the highest frequency while FF in the next highest in the majority of cases, the exceptions being the urban high caste Bengali females and the Mundari males. In both the latter cases, the heterotype TF occurs in the next highest frequency. If, however, the two heterotypes TF and FT are added together the second position of FF is considerably altered in the majority of the samples. The high frequency of 23.08 per cent of the homotype FF in the rural female Bengali Brahmans, however, stands in sharp distinction from 2.0 per cent of the same type in the urban high caste female Bengalis. The homotype OO appears to be very rare—only 6 instances have been found in course of the present study. Hawkes did not find any instance of the heterotypes of O in England. It is also a rare type in this country.

## HEREDITY OF THE FOOT TYPES

The following pedigrees (A, 1-12) were collected in course of the morphological studies of the foot.

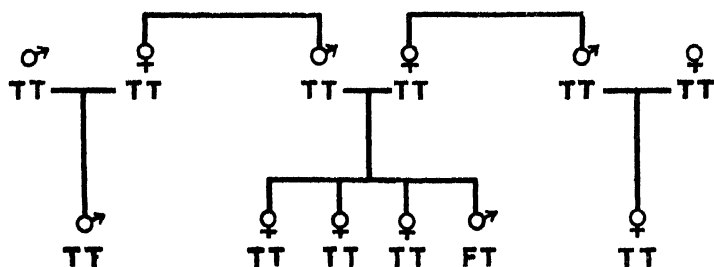


## PEDIGREES A

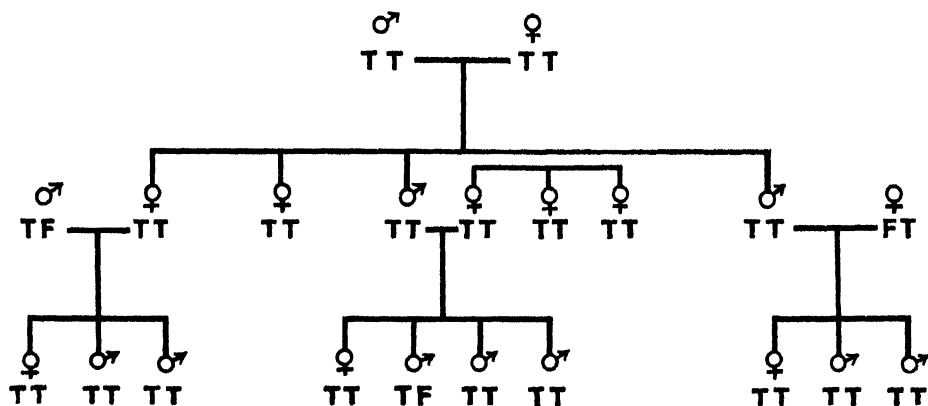
For facilities of comparison Hawkes' pedigrees (B) have been converted into the symbols followed in the present paper and are reproduced below.

### HAWKES DATA (1912)

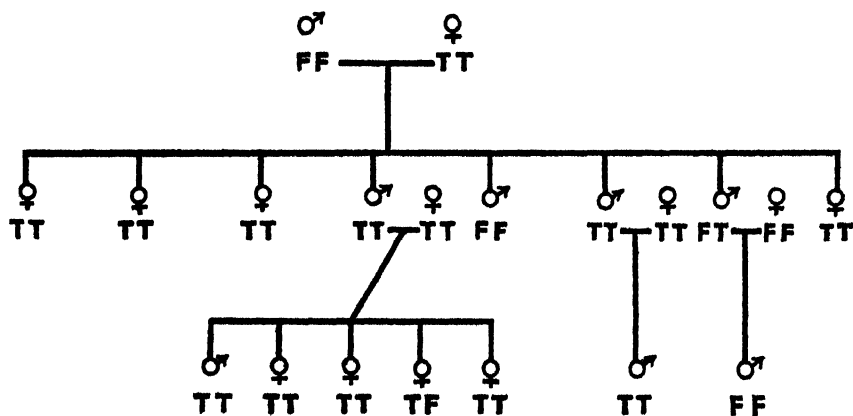
#### TREE B



#### TREE C



#### TREE D

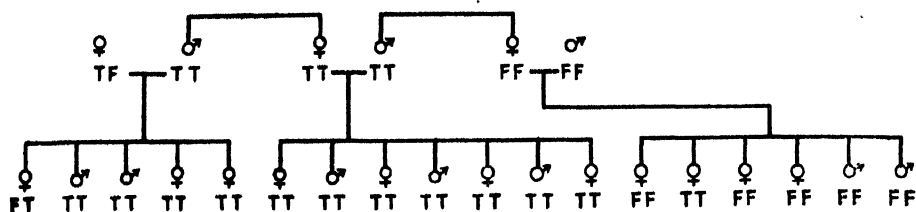


#### PEDIGREES B

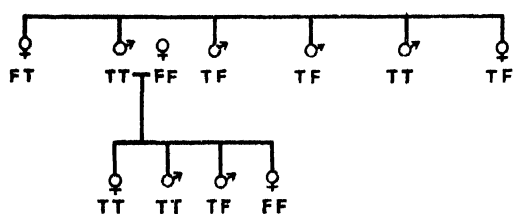


## HAWKES' DATA (1912)

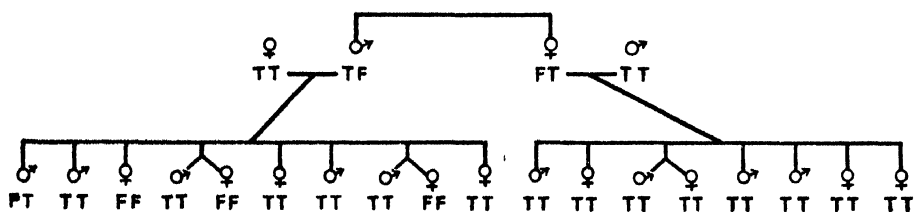
TREE E



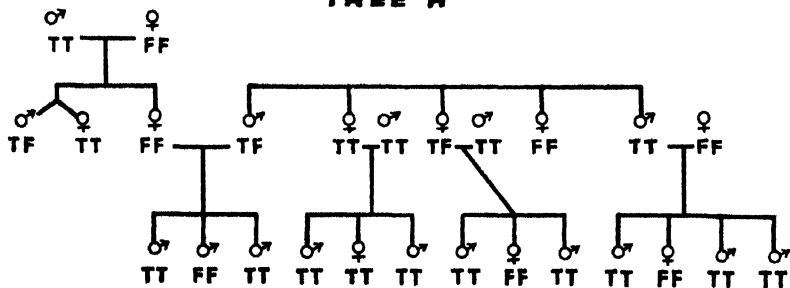
TREE F



TREE G



TREE H



## PEDIGREES B

It will be seen from the above pedigrees that when TT×TT are crossed there are always chances of having TT children, which indicate that this type breeds true. An exception is, however, met with in Pedigree 5 where an FT child is born out of such a union. This child was found at the time of first enquiry to have the second toe of his left foot longer than the first. He was investigated again at the time of writing when he was found to belong to TT. The complete data are as follows :

| Date     | Age | Left |       | Right |     |
|----------|-----|------|-------|-------|-----|
|          |     | I    | II    | I     | II  |
| 22-12-54 | 12  | 203  | 203.5 | 202   | 198 |
| 21-2-57  | 14  | 237  | 231   | 236   | 228 |

There are thus chances of young children showing a different picture with the increase of age. This solitary example, however, goes against the observation of Hawkes who suggested that the "adult condition as regards toe type is reached by the age of two years". As a matter of fact, the present writer excluded all children below two years of age on the basis of the above suggestion of Hawkes.

Hawkes has also found 4 instances of TF or FT children in TT×TT matings, but these four exceptional cases were not investigated after a certain interval. Their ages are also unknown.

The dominance of TT over the other phenotypes FF, FT, OO, etc., is however, apparent. In Pedigree 3, though the mating TT×FF has resulted into all TT children, there are possibilities of FF or FT types, as will be evident from the data of Hawkes. Even an OO type has been found to occur in the above mating. Hawkes has recorded 25 matings of the above type and suggested a partial dominance approximating to 2.75 : 1. Data for FF×FF matings are too few. Hawkes found only two instances and the present author only one (Ped. 5). Hawkes found one TT child in a total of 9 children while the present data show one child each of TT and FF.

The matings involving the heterotype FT are only 2 in number (Ped. 6a and 12) and in the mating with each of the homotypes TT and FF an O child has come out. Both these two children are below 10 years of age.

Hawkes did not find any case of combinations of the O type and as such her pedigree material is not complicated with this genetic factor. The present writer has found all the three adult combinations OO, OT and OF and in the two Pedigrees 7 and 11, two matings with adult children are seen :

$$\text{♂ OF} \times \text{♀ FT} - \text{FF } \text{♀}$$

$$\text{♂ TO} \times \text{♀ FF} - \text{FO } \text{♂}$$

It will be apparent from the above scanty data that O also behaves hereditarily and the dominance of T over it is also indicated.

It is, however, worthwhile to point out that in the study of the heredity of the foot types or in the collection of pedigrees, sufficient time should be allowed for the completion of growth in the foot of the children. It appears that Hawkes' estimate of 2 years is probably too low, and children below 14 years of age cannot always be reliably taken into account. Growth studies, however, are necessary to arrive at the exact figure at which the child's foot ceases to grow.

# INTERSPACES

A study of the interspaces between the toes shows the following 11 types (Fig. 1). They are :

1. Coalescent
2. Slit-like
3. Tubular
4. Bulbous      4a. circular base;    4b. triangular base.
5. Rectangular
6. V form      6a. elongated ;      6b. constricted.
7. U form      7a. wide ;      7b. constricted.
8. Y form
9. Hooked
10. M form
11. Pointed

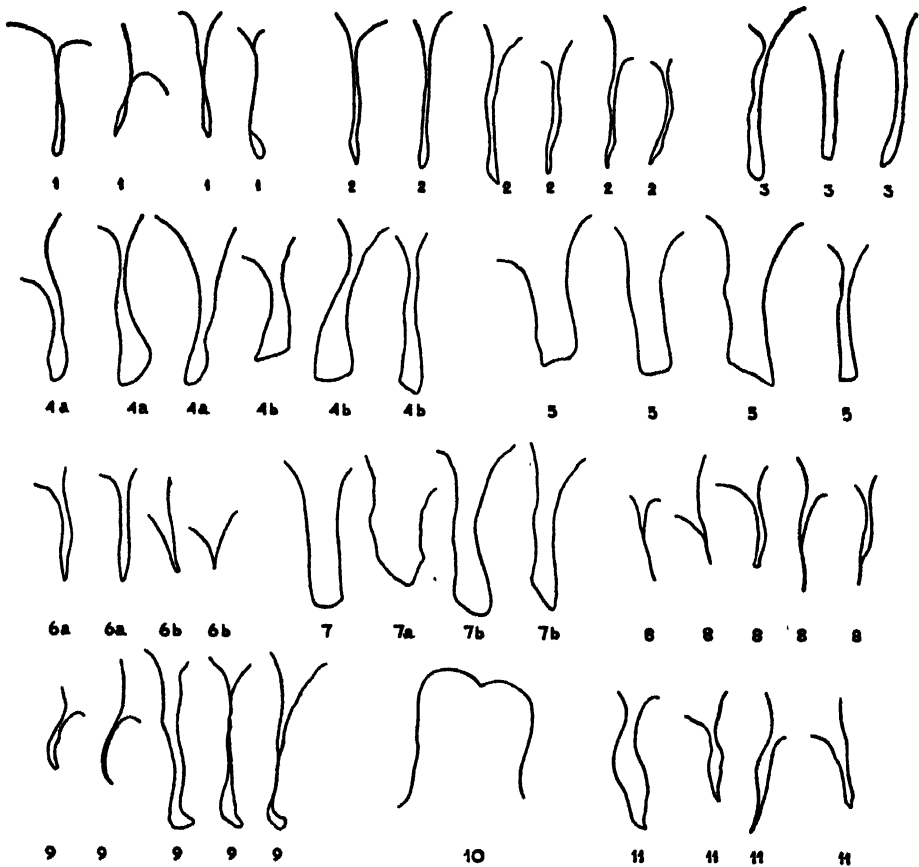


Fig. 1. Forms of interspaces.

The distribution of the above 11 types has been studied in the two sexes of two populations only—(i) Urban Bengali and (ii) Rural Bengali Brahmans. Sexual difference also appears to be very much marked in this character. It has been found that in both the rural and urban populations the females, as a whole, show more interspaces between the toes than the males. It will be evident from the following table (Table V) extracted out of Tables III and IV,

TABLE III  
Percentage Frequency of Interspace Forms (cf. Fig. 1)  
(Urban Bengali)

| Types | male  |       |       |       |       |       |       |       |     |       | female |      |      |      |      |      |      |      |      |     |       |   |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|-------|--------|------|------|------|------|------|------|------|------|-----|-------|---|
|       | Lt.   |       |       |       |       | Rt.   |       |       |     |       | Lt.    |      |      |      |      | Rt.  |      |      |      |     |       |   |
|       | I     | II    | III   | IV    |       | I     | II    | III   | IV  | Total | %      | I    | II   | III  | IV   |      | I    | II   | III  | IV  | Total | % |
| 0     | 43    | 92    | 91    | 111   | 48    | 98    | 105   | 114   | 702 | 75.65 |        | 8    | 25   | 23   | 35   | 7    | 21   | 27   | 39   | 185 | 46.25 |   |
| %     | 37.07 | 79.31 | 78.45 | 95.69 | 41.27 | 84.48 | 90.52 | 98.27 |     |       |        | 16.0 | 50.0 | 46.0 | 70.0 | 14.0 | 42.0 | 54.0 | 78.0 |     |       |   |
| 1     | 6     | 1     | 2     | -     | 6     | 2     | -     | -     | 17  | 1.83  |        | -    | 1    | 2    | 3    | 1    | -    | 1    | 1    | 9   | 2.25  |   |
| 2     | 5     | 2     | 2     | -     | 4     | 2     | 1     | -     | 16  | 1.72  |        | -    | 1    | -    | -    | -    | 3    | 1    | 2    | 7   | 1.75  |   |
| 3     | 9     | 1     | 2     | -     | 10    | 2     | -     | -     | 24  | 2.59  |        | 1    | 3    | 5    | -    | 5    | 4    | 6    | 1    | 25  | 6.25  |   |
| 4a    | 16    | 1     | 2     | -     | 21    | -     | 1     | 1     | 42  | 4.53  |        | 16   | 4    | 3    | -    | 17   | 5    | 5    | 1    | 51  | 12.75 |   |
| 4b    | 13    | -     | -     | -     | 14    | -     | 1     | -     | 28  | 3.02  |        | 5    | 1    | 1    | 1    | 3    | 1    | -    | -    | 12  | 3.00  |   |
| 5     | 3     | -     | -     | -     | 1     | -     | -     | -     | 4   | 0.43  |        | 7    | -    | 1    | -    | 6    | -    | -    | -    | 14  | 3.50  |   |
| 6     | 3     | 2     | 8     | 1     | 2     | 2     | 1     | 1     | 20  | 2.16  |        | 2    | -    | 3    | 3    | 1    | -    | 2    | 1    | 12  | 3.00  |   |
| 6a    | 1     | 2     | 1     | -     | -     | 3     | 1     | -     | 8   | 0.86  |        | -    | 2    | 3    | -    | 1    | 2    | -    | -    | 8   | 2.00  |   |
| 6b    | -     | 1     | 2     | -     | 1     | -     | 1     | -     | 5   | 0.54  |        | -    | 1    | 1    | 1    | -    | 1    | 2    | -    | 6   | 1.50  |   |
| 7     | 2     | 2     | 2     | -     | 4     | -     | -     | -     | 10  | 1.08  |        | 2    | -    | -    | -    | 1    | -    | 1    | -    | 4   | 1.00  |   |
| 7a    | 1     | -     | -     | 2     | 1     | -     | -     | -     | 4   | 0.43  |        | 1    | -    | -    | -    | 1    | -    | -    | -    | 2   | 0.50  |   |
| 7b    | 8     | 3     | 2     | 1     | 3     | 1     | 2     | -     | 20  | 2.16  |        | 6    | 1    | 2    | -    | 7    | 1    | 2    | -    | 19  | 4.75  |   |
| 8     | 4     | 2     | -     | -     | -     | 1     | 2     | -     | 9   | 0.97  |        | 2    | 1    | 5    | 4    | -    | 3    | 2    | 2    | 19  | 4.75  |   |
| 9     | 1     | -     | -     | 1     | 1     | 1     | -     | -     | 4   | 0.43  |        | -    | -    | -    | 2    | -    | 1    | -    | 3    | 6   | 1.50  |   |
| 10    | -     | 5     | -     | -     | -     | 2     | -     | -     | 7   | 0.75  |        | -    | 7    | -    | -    | -    | 4    | 1    | -    | 12  | 3.00  |   |
| 11    | 1     | 2     | 2     | -     | -     | 2     | 1     | -     | 8   | 0.86  |        | -    | 3    | 1    | 1    | -    | 4    | -    | -    | 9   | 2.25  |   |
|       | 116   | 116   | 116   | 116   | 116   | 116   | 116   | 116   | 928 |       |        | 50   | 50   | 50   | 50   | 50   | 50   | 50   | 50   | 400 |       |   |

TABLE IV  
Percentage Frequency of Interspace Forms (cf. Fig. 1)  
(Rural Bengali Brahmans)

| Types | male  |       |       |       |       |       |      |       |     |       | female |    |      |      |       |       |      |       |       |    |       |   |
|-------|-------|-------|-------|-------|-------|-------|------|-------|-----|-------|--------|----|------|------|-------|-------|------|-------|-------|----|-------|---|
|       | Lt    |       |       |       |       | Rt    |      |       |     |       | Total  |    |      |      |       | %     |      |       |       |    |       |   |
|       |       |       |       |       |       |       |      |       |     |       |        |    |      |      |       |       |      |       |       |    |       |   |
|       | I     | II    | III   | IV    | I     | II    | III  | IV    | I   | II    | III    | IV | I    | II   | III   | IV    | I    | II    | III   | IV | Total | % |
| 0     | 17    | 32    | 40    | 40    | 26    | 35    | 39   | 43    | 272 | 52.31 |        |    |      |      |       |       |      |       |       |    |       |   |
| %     | 26.15 | 49.24 | 61.54 | 61.54 | 40.00 | 53.85 | 60.0 | 66.15 |     |       |        |    | 7.69 | 7.69 | 34.62 | 11.53 | 7.69 | 11.53 | 34.62 |    |       |   |
| 1     | 6     | 11    | 7     | 5     | 3     | 13    | 8    | 4     | 57  | 10.96 |        |    |      |      |       |       |      |       |       |    |       |   |
| 2     | 2     | 7     | -     | -     | 5     | 3     | 2    | -     | 19  | 3.65  |        |    |      |      |       |       |      |       |       |    |       |   |
| 3     | 2     | 2     | 2     | -     | 1     | 2     | 3    | -     | 12  | 2.31  |        |    |      |      |       |       |      |       |       |    |       |   |
| 4a    | 8     | -     | 1     | 1     | 8     | -     | 1    | -     | 19  | 3.65  |        |    |      |      |       |       |      |       |       |    |       |   |
| 4b    | 15    | -     | 4     | 3     | 10    | 1     | 3    | 2     | 38  | 7.31  |        |    |      |      |       |       |      |       |       |    |       |   |
| 5     | 2     | -     | -     | 1     | 3     | -     | -    | 1     | 7   | 1.35  |        |    |      |      |       |       |      |       |       |    |       |   |
| 6     | -     | -     | -     | -     | -     | -     | -    | -     | -   | -     |        |    |      |      |       |       |      |       |       |    |       |   |
| 6a    | 1     | 1     | 1     | 1     | -     | -     | -    | -     | 4   | 0.77  |        |    |      |      |       |       |      |       |       |    |       |   |
| 6b    | -     | 1     | 1     | -     | -     | 1     | -    | 1     | 4   | 0.77  |        |    |      |      |       |       |      |       |       |    |       |   |
| 7     | 1     | 1     | 1     | 1     | 3     | -     | -    | 1     | 8   | 1.54  |        |    |      |      |       |       |      |       |       |    |       |   |
| 7a    | 3     | -     | -     | -     | 1     | -     | -    | 1     | 5   | 0.96  |        |    |      |      |       |       |      |       |       |    |       |   |
| 7b    | 4     | 1     | -     | 1     | 1     | -     | -    | -     | 7   | 1.35  |        |    |      |      |       |       |      |       |       |    |       |   |
| 8     | 1     | 3     | 5     | 4     | 2     | 3     | 5    | 3     | 26  | 5.00  |        |    |      |      |       |       |      |       |       |    |       |   |
| 9     | 3     | 1     | 1     | 8     | 2     | 1     | 2    | 9     | 27  | 5.19  |        |    |      |      |       |       |      |       |       |    |       |   |
| 10    | -     | 5     | 1     | -     | -     | 4     | 2    | -     | 12  | 2.31  |        |    |      |      |       |       |      |       |       |    |       |   |
| 11    | -     | -     | 1     | -     | -     | 2     | -    | -     | 3   | 0.58  |        |    |      |      |       |       |      |       |       |    |       |   |
|       | 65    | 65    | 65    | 65    | 65    | 65    | 65   | 65    | 520 |       |        |    | 26   | 26   | 26    | 26    | 26   | 26    | 26    | 26 | 208   |   |

TABLE V  
*Frequency of Interspaces (in %)*

| Characters                   | Urban Bengali |        | Rural Bengali Brahmans |        |
|------------------------------|---------------|--------|------------------------|--------|
|                              | male          | female | male                   | female |
| No interspace in either foot | 21.55         | 4.0    | 16.92                  | 0      |
| „ „ „ right „                | 12.07         | 2.0    | 10.77                  | 0      |
| „ „ „ left „                 | 5.17          | 2.0    | 1.54                   | 0      |
| „ „ „ per „                  | 30.17         | 6.0    | 23.08                  | 0      |

It will be apparent from Table V that the urban Bengali females show a significantly low percentage of the absence of interspaces between the toes than the urban males, while in the case of rural females this character of the foot is present in all. The interspaces, therefore, appear to be more a characteristic of the female foot than the male foot.

As regards the other interspaces, the I interspace (between hallux and the II toe) occurs in the highest percentage, excepting for the rural females, among whom the interspaces I-III occur in almost equal percentages. The IV interspace (between the IV toe and the little toe) occurs in the least frequency in all excepting the rural females. A wide variability is seen in the two sexes and also between the rural and urban samples. The detailed data for the left and right sides combined together are given in the Table VI below.

TABLE VI  
*Frequency of Interspaces (Rt. + Lt., in %)*

| Rural            |       |       |       |       |        |       |       |       |
|------------------|-------|-------|-------|-------|--------|-------|-------|-------|
| Types            | male  |       |       |       | female |       |       |       |
|                  | I     | II    | III   | IV    | I      | II    | III   | IV    |
| " No "           | 33.08 | 51.55 | 60.77 | 63.35 | 9.61   | 7.69  | 9.61  | 34.62 |
| Others (Fig. 1.) | 66.92 | 48.45 | 39.23 | 36.65 | 91.39  | 92.31 | 90.39 | 65.38 |

| Urban            |       |       |       |       |        |      |      |      |
|------------------|-------|-------|-------|-------|--------|------|------|------|
| Types            | male  |       |       |       | female |      |      |      |
|                  | I     | II    | III   | IV    | I      | II   | III  | IV   |
| " No "           | 39.17 | 81.90 | 84.49 | 96.88 | 15.0   | 46.0 | 50.0 | 74.0 |
| Others (Fig. 1.) | 60.83 | 18.10 | 15.51 | 3.12  | 85.0   | 54.0 | 50.0 | 26.0 |

In urban males the I interspace occurs in 60.83 per cent while in the females it is found in 85 per cent. In rural males and females the same is found in 66.92

TABLE  
*Statistical Constants*

| Sr.<br>no. | Caste or Tribe        | No. | Foot Length (mm) |                   | Foot Breadth (mm) |                   |
|------------|-----------------------|-----|------------------|-------------------|-------------------|-------------------|
|            |                       |     | Range            | Mean $\pm$ S.E.   | Range             | Mean $\pm$ S.E.   |
| $\sigma$   |                       |     |                  |                   |                   |                   |
| 1.         | Bengal Low Castes     | 160 | 216-282.5        | 244.35 $\pm$ 0.89 | 84-110.5          | 95.44 $\pm$ 0.42  |
| 2.         | Bengal Artisan Castes | 134 | 217-285          | 248.30 $\pm$ 1.08 | 84-113            | 95.80 $\pm$ 0.46  |
| 3.         | Bengal Muslims        | 54  | 219.5-258        | 241.70 $\pm$ 1.46 | 86-113            | 96.89 $\pm$ 0.73  |
| 4.         | „ Rural Brahmans      | 130 | 213-279          | 246.25 $\pm$ 1.01 | 75-109            | 94.78 $\pm$ 0.51  |
| 5.         | „ Urban High Castes   | 240 | 220-284          | 253.30 $\pm$ 0.73 | 84-113            | 98.53 $\pm$ 0.36  |
| 6.         | Juang                 | 86  | 211.5-268        | 241.95 $\pm$ 1.25 | 85.5-118          | 95.90 $\pm$ 0.62  |
| 7.         | Oraon                 | 88  | 225-279.5        | 259.90 $\pm$ 1.12 | 84-116.5          | 101.20 $\pm$ 0.69 |
| 8.         | Pahira                | 58  | 191-257          | 229.70 $\pm$ 1.60 | 75-109            | 92.80 $\pm$ 0.93  |
| 9.         | Mundari               | 90  | 227-274          | 251.20 $\pm$ 1.14 | 88-115            | 100.30 $\pm$ 0.65 |
| 10.        | Vedda                 | 26  | 173-249          | 230.00 $\pm$ 2.65 | 71-103            | 85.34 $\pm$ 1.45  |
| 11.        | Abor                  | 84  | 212-274          | 241.05 $\pm$ 1.09 | —                 | —                 |
| $\phi$     |                       |     |                  |                   |                   |                   |
| 1.         | Abor                  | 10  | 219-239          | 228.3             | —                 | —                 |
| 2.         | Mundari               | 18  | 198-253          | 227.0             | 86.5-97           | 90.94             |
| 3.         | Beng. Rural Brahmans  | 52  | 209-247          | 228.80 $\pm$ 1.25 | 77-96             | 86.61 $\pm$ 0.55  |
| 4.         | „ Urban High Castes   | 100 | 205-251          | 226.40 $\pm$ 1.08 | 72-106            | 87.13 $\pm$ 0.66  |

## VII

*of Foot*

| Leng.       | Br. Index of Foot | Hallux Divergence Angle |                  | Author        |
|-------------|-------------------|-------------------------|------------------|---------------|
|             |                   | Range                   | Mean $\pm$ S.E.  |               |
| 35.66-44.17 | 39.77 $\pm$ 0.17  | 4.5°-10°                | 5.87° $\pm$ 0.16 | Present study |
| 34.41-44.76 | 38.72 $\pm$ 0.15  | 4°-9°                   | 6.76° $\pm$ 0.17 | „             |
| 37.37-45.82 | 40.17 $\pm$ 0.27  | 5°-8.5°                 | 6.72° $\pm$ 0.23 | „             |
| 33.01-43.30 | 38.62 $\pm$ 0.17  | 4.5°-10.5°              | 6.42° $\pm$ 0.20 | „             |
| 34.68-45.00 | 39.00 $\pm$ 0.11  | 4°-9°                   | 6.48° $\pm$ 0.13 | „             |
| 36.12-45.12 | 39.73 $\pm$ 0.20  | 4°-10°                  | 5.85° $\pm$ 0.20 | „             |
| 35.96-44.23 | 39.83 $\pm$ 0.20  | 4.5°-9°                 | 6.68° $\pm$ 0.22 | „             |
| 36.91-44.21 | 40.40 $\pm$ 0.26  | 5.5°-10.5°              | 5.98° $\pm$ 0.27 | „             |
| 32.37-43.98 | 39.98 $\pm$ 0.23  | 4.5°-9.5°               | 6.26° $\pm$ 0.22 | „             |
| 31.42-41.37 | 36.46 $\pm$ 0.41  | —                       | —                | Osman Hill    |
| —           | —                 | —                       | —                | Kemp          |
|             |                   | —                       | —                | Kemp          |
| 36.95-43.94 | 39.67             | —                       | —                | Present study |
| 34.70-41.86 | 37.89 $\pm$ 0.32  | 4.5°-10°                | 5.60° $\pm$ 0.24 | „             |
| 32.58-44.29 | 38.57 $\pm$ 0.23  | 4.5°-10°                | 6.70° $\pm$ 0.25 | „             |



per cent and 91.39 per cent respectively. Correspondingly the IV interspace occurs in urban males and females in 3.12 per cent and 26 per cent respectively while in the rural Bengali Brahmans it is seen in 36.65 per cent and 65.38 per cent in males and females respectively.

The II and III interspaces appear in almost equal frequencies. They are found in 18.10 per cent and 15.51 per cent respectively in urban males ; in 54 per cent and 50 per cent respectively in urban females ; in 48.45 per cent and 39.23 per cent respectively in rural males and in 92.31 per cent and 90.39 per cent respectively in rural females.

It will be apparent, therefore, that a wide sexual variation is present in all the four interspaces while the rural-urban variation is manifested mostly in the three interspaces II, III and IV. The I interspace shows the least of it.

The rural percentages are always higher than the urban, particularly in the case of II-IV interspaces ; only in the case of I interspace do they nearly agree with one another. The rural-urban difference also appears to be higher in the case of males than the females and in the case of the IV interspace, the rural value is 12 times that of the urban. The position of the little toe may here be mentioned. It was observed in the case of a large number of urban samples that the little toe does not touch the ground but appears as an appendage at a slightly higher level and directed upwards. This upward direction of the toes is also more a character of the rural people than that of the urban, whose toes show a tendency to curve medially and downwards, particularly in the case of the IV and the V toes.

Wood Jones has pointed out that the little toe "suggests phylogenetic decrepitude" and the wearing of the boots has nothing to do with its general anatomical condition. Its structural changes are due to the demand of "functional requirements" and it is not a "degenerating member" of the human foot. He, however, mentions that "the boots of civilization often serve it badly". Whether the wearing of tight shoes in childhood, as is seen commonly in the cities, has any effect in shaping the toes or not, they are probably effective in obliterating or lessening the natural interspaces. Urban school children, below the ages of 12, show a tendency to wear tight-fitting shoe, so that the whole foot is encased as a compact whole. The rural-urban difference in the frequency of the interspaces, II, III and IV may have its origin in the use of footwear, which is commoner in the cities than in the villages. Shoes are a handicap in the villages, both from the social and ecological point of view.

The different forms of interspaces (Fig. 1) also show very clearly the sexual and the rural-urban variation. In urban males, the absence of interspaces occurs in 75.65 per cent (Table III) which renders the other forms of interspaces almost inconspicuous—the highest percentage of 4.53 is met with in the form 4a (bulbous with circular base). The next highest form is seen in 4b (bulbous with triangular base) with a percentage of 3.02. The rural males, on the other hand, show the absence of interspace in 52.31 per cent (Table IV) and the interspace form 1 (coalesced) occurs in the highest percentage of 10.96. The next highest percentage is seen in the form 4b (7.31 per cent). There is, however, a high element of the bulbous form in the urban sample, which is also shared by the rural people in addition to the coalesced form (type 1, Fig. 1). The bulbous form of interspace is also predominant among the females, both urban (15.75 per cent) and rural (18.27 per cent). The next highest form is seen in the types 3 and 7, which occur interchangeably in the two female samples. In urban females, types 3 and 7 occur in the equal percentage of 6.25, while in the rural sample the former type appears in 6.73 per cent and the latter in 15.39 per cent.

These observations are in harmony with our previous remark that the rural-urban difference is greater in the males than in the females. We have also shown

that the female foot shows more interspaces than the male foot. The cause of the rural-urban variation in the interspaces of the male foot probably lies, as already mentioned, in the footwear. The coalesced type of interspace may be easily obliterated due to a tight shoe and the male rural frequency of 10.96 per cent has been reduced to 1.83 per cent in the city. On the other hand, the bulbous type of interspace is probably the least affected by a footwear. The latter might cause the two toes to touch each other at the upper end due to lateral pressure, but the lower bulbous space, which is formed by the two lateral depressions at the base of the toe, almost remains unaltered. The rural-urban difference in the bulbous type is not so great as in the case of the coalesced type (1); it is 7.55 per cent for urban and 10.96 per cent for rural.

The higher frequency of interspaces in the females than in the males might be associated with the same sexual relationship in respect of the F type of foot, with its II toe longer than the hallux. Both the characters behave hereditarily and only further researches can lead us to useful conclusions of applied value. The Police authorities now retain foot prints along with finger prints for all undertrial prisoners. It will be useful to retain a foot contour as well with all interspaces, since the peculiarities of the toes and the interspaces may provide additional information for identification.

#### HEREDITY OF INTERSPACES

The form of the interspaces also appears to be hereditary. The presence of interspaces appears to be dominant over "no interspaces" as will be apparent from the interspaces of Pedigree 4 (Fig. 2).

Similarly, in Pedigree 1, while both the parents have interspaces, they are somewhat marked in the mother and the M form (Fig. 1, No. 10) of interspace (Fig. 3), which has also been found in other members of the mother's family, is inherited in the children. Other pedigrees also bear out the heredity of this M form of interspace.

#### ANTHROPOMETRIC CHARACTERS

The anthropometric measurements of the foot comprise: (1) foot length, (2) foot breadth and (3) hallux divergence angle. They are shown in Table VII along with the length-breadth index of foot, calculated from (1) and (2) given above. All the above measurements are taken on the outline (Figs. 4 and 5). In measuring the foot length, the greatest length has always been taken into account. A 300 mm. long sliding clipper was used for this purpose.

The statistical constants of the foot of the various peoples are given in Table VII.

It will be apparent from the above Table that the Pahiras ( $229.70 \pm 1.60$ ) of Dalma Hills, Manbhum show the smallest length, closely followed by the Veddass ( $230.0 \pm 2.65$ ) of Ceylon, while the Oraons ( $259.90 \pm 1.12$ ) show the greatest length of all. The Oraons also show the highest breadth of all ( $101.20 \pm 0.69$ ) while the lowest breadth is seen among the Veddass ( $85.34 \pm 1.45$ ). The Pahiras rank next to the Veddass in foot breadth ( $92.80 \pm 0.93$ ). Thus, both in length and breadth the Pahiras appear to be closer to the Veddass than the other groups—a fact which is also confirmed by other studies. The Oraons appear to be closer to the Mundas in foot breadth only; in foot length they stand wide apart.

The male rural Brahmans and the urban high castes show significant differences both in the length and breadth of the foot, while the females of the two groups appear to be close to one another. The rural females show a slightly greater length and lesser breadth than do the urban and they appear to be significantly similar to one another ('t' for length = 1.45; 't' for breadth = 0.60).

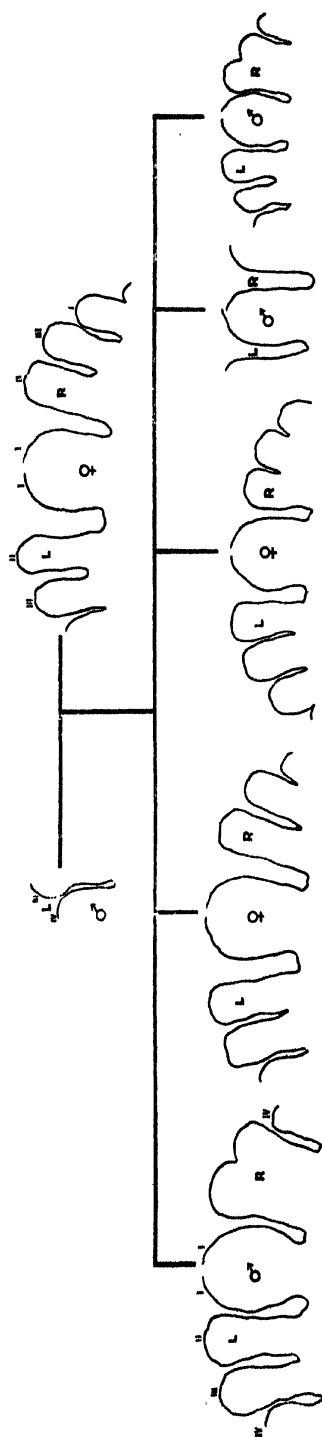


Fig. 2. Pedigree showing heredity of interspaces.

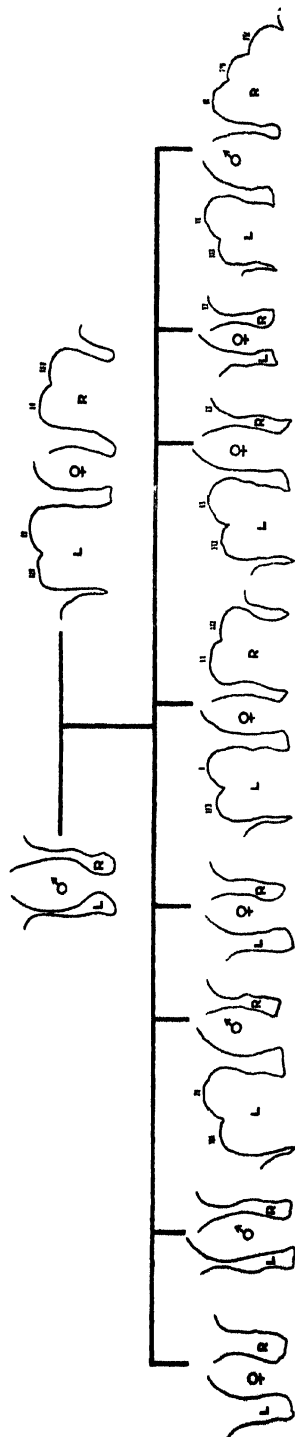
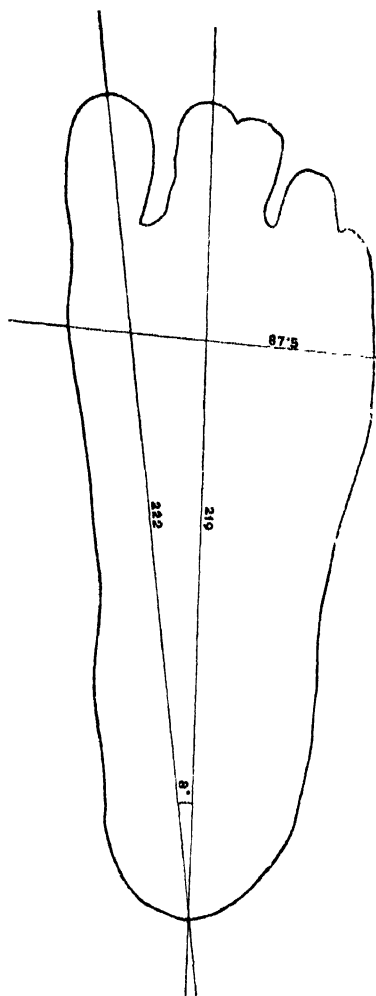
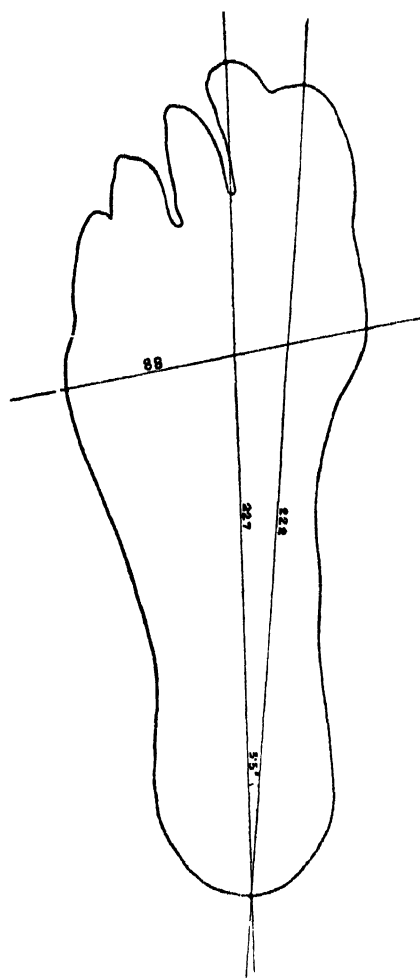


Fig. 3. Pedigree showing heredity of interspace (M from)

Fig. 4. Contour of foot type T ( $1 > 2$ ).Fig. 5. Contour of foot type F ( $2 > 1$ ).

The differences, found in respect of the absolute length and breadth of foot, are, however, evened out when the length-breadth index of foot is taken into consideration. In absolute length and breadth the rural Brahmans and the urban high castes were found to differ significantly, the values of 't' have been found to be 5.64 and 6.25 respectively, whereas in respect of the index, the value of 't' is 1.90, indicating a close relationship between the two. The females of the above two groups show the value of 't' to be 1.74 in respect of the above index, whereas the same for the length and breadth are 1.45 and 0.60 respectively. Similarly, the Mundari group shows an affinity in respect of this index, with the other aboriginal groups, Juang ('t' = 0.83), Oraon ('t' = 0.50) and Pahira ('t' = 1.20). They show close affinity with the Muslims ('t' = 0.53) and the Bengal low castes ('t' = 0.72). The latter caste group also shows close affinity with the Muslims ('t' = 1.25), Juang ('t' = 0.15) and Oraon ('t' = 0.23).

In the hallux divergence angle the least divergence of  $5.85 \pm 0.20^\circ$  has been found among the Juangs while the highest divergence is seen among the rural artisan castes of Bengal ( $6.76 \pm 0.17^\circ$ ). The range of variability does not appear to be great in the different samples studied in this paper.

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# ON THE STRUCTURE AND LIFE-HISTORY OF A NEW SPECIES OF *ANABAENA*

(*A. RANDHAWAE* SP. NOV.)\*

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(Communicated by M. S. Randhawa, F.N.I.)

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## ABSTRACT

A detailed account of the structure and life-history of a new species of *Anabaena* (*A. Randhawae* sp. nov.) has been given here. The alga is characterised by terminal heterocysts and spherical akinetes which are usually remote from the heterocysts but are extremely variable in position.

## INTRODUCTION

The object of the present communication is to describe the structure and life-history of a new and interesting form of *Anabaena*, which was found free-floating in one of the rain-water puddles on 5th January, 1957, inside the Indian Agricultural Research Institute grounds, New Delhi, India.

## GENERAL MORPHOLOGY

The plant body consists of a broad mucilagenous expanse, the outer layer of which is firmer than the inner, enclosing numerous trichomes. Very rarely, an individual sheath is also discernible around some trichomes, after prolonged immersion in the aqueous methylene blue (Text-fig. 2).

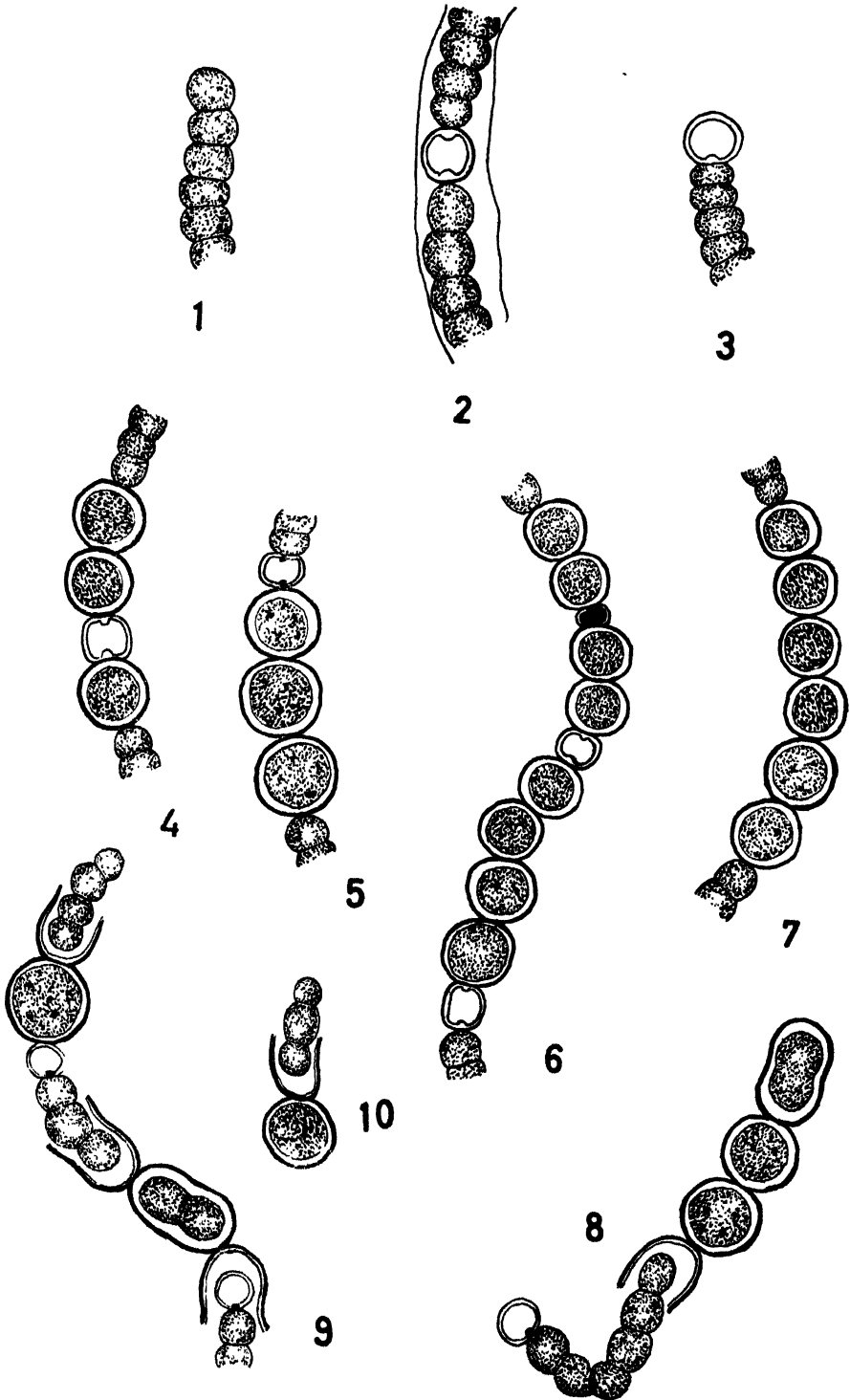
The cells are barrel-shaped,  $3.8-4.7\mu$  broad and  $3.8-5.7\mu$  long. The cross wall regions are constricted. The cell contents are coarsely granular and blue green in colour. The cell wall is coloured light blue after treating with iodine and sulphuric acid. The end cell is rounded (Text-fig. 1).

The heterocysts are usually intercalary, but also occasionally found to occupy the terminal position and in such cases the heterocysts are terminal at one end of the trichome only (Text-fig. 3; Pl. XX. Fig. 1). The contents are homogenous and pale blue green in colour. The heterocysts are more or less barrel-shaped with flat ends or sometimes spherical in shape;  $5.7-6.6\mu$  broad and  $4.7-6.6\mu$  long. The wall of the heterocysts shows the usual deep blue colouration with iodine and sulphuric acid.

During akinete formation, any vegetative cell which is to form an akinete, becomes richer in contents, increases in size and finally develops a thick wall around it, while the other adjoining cells maintain their original barrel-shape. The akinetes are usually formed remote from the heterocysts (Text-fig. 7), but occasionally they are contiguous to the heterocysts also, viz., one on either side or one to many

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\* Named after Dr. M. S. Randhawa, Vice-President, I.C.A.R., for his valuable contributions to the field of Algology.



Text-figs. 1-10. *Anabaena randhawae* sp. nov. Fig. 1, portion of a trichome showing the spherical end cell; fig. 2, part of the trichome enclosed by an individual sheath; fig. 3, trichome with a terminal heterocyst; figs. 4-6, akinetes contiguous to heterocysts; fig. 7, akinetes remote from heterocyst; figs. 8-10, different stages of germination of the akinetes. (All figures under  $\times 1600$ ).

on one side only or a short chain on either side of the heterocysts (Text-figs. 4, 5, 6). The contents of the mature akinetes are granular, occupying the whole protoplast. The outer envelope is thick, smooth and dark brown in colour. The akinetes are spherical,  $5.7-7.6\mu$  in diameter.

#### GERMINATION OF THE AKINETES

The akinetes were found to germinate *in situ* (Text-figs. 8-10; Pl. XX. Figs. 2-5). Prior to germination, the contents of the akinetes contract from the wall and in some cases the akinetes themselves slightly elongate with a slight median constriction (Text-figs. 8, 9). The contents divide transversely into two to form a two-celled germling (Text-fig. 9; Pl. XX. Fig. 2), which later undergoes further transverse divisions to form a trichome. The wall of the akinete was found to thin off at the point of rupture in the median portion and the dissolution of the akinete wall at this region may be probably due to some enzymatic action although a mechanical stretching force may also be operative due to the increasing number of the cells of the germling. Germlings up to four-celled stages were clearly seen inside the mother akinete wall (Pl. XX. Figs. 3, 4). In some cases the terminal cell of the germling is transformed into a heterocyst thereby arresting the further development of the trichome on that side (Text-figs. 9, 10).

#### SYSTEMATIC POSITION

The present form in its more or less straight trichomes and variable position of the akinetes resembles *A. wernerii* Brunn., *A. scheremetievi* Elenk., and *A. planktonica* Brunn., but differs from the first in its much narrower trichomes, heterocysts and akinetes, in the absence of pseudovacuoles, in the occasional terminal heterocysts. *A. scheremetievi* differs from the present form in its S-forming trichomes, broader trichomes, occasional ellipsoidal akinetes and trichomes enclosed within a broad mucilage. *A. planktonica* differs in having a broad mucilage, smaller cells, planktonic habit and in the absence of terminal heterocysts. This form resembles *A. sphaerica*, *A. fertilissima*, *A. spiroides*, *A. gelatinicola* and *A. anomala* in possessing spherical akinetes but differs from all mainly in having akinetes extremely variable in position and in possessing terminal heterocysts. The presence of terminal heterocysts brings this form near to *A. oryzae* Fritsch, but the latter differs in having the akinetes characteristically next to the terminal heterocysts and in the attenuating trichomes with a conical terminal heterocyst.

The present form may, therefore, be regarded as a new species of *Anabaena* and it is proposed to name it as *Anabaena randhavae* sp. nov.

#### DIAGNOSIS

##### *Anabaena randhavae* sp. nov.

Trichomes more or less straight, embedded in a broad mucilage; trichomes with occasional terminal spherical heterocyst at one end of the trichome; cells barrel-shaped,  $3.8-4.7 \times 3.8-5.7\mu$ ; heterocysts barrel-shaped to spherical,  $5.7-6.6 \times 4.7-6.6\mu$ ; akinetes spherical, usually remote from the heterocysts, less commonly contiguous to the heterocysts;  $5.7-7.6\mu$  in diameter.

Habitat: Free-floating in a rain water puddle inside the Indian Agricultural Research Institute grounds, New Delhi, India, January 5, 1957.

##### *Anabaena randhavae* sp. nov.

Trichomata plus minusve recta, immersa in gluten copiosum trichomata nonnumquam ad unum apicem ornata heterocystis singulis; cellulae doliiformes,



3.8–4.7 × 3.8–5.7 $\mu$ ; heterocysta doliiformia vel sphaerica, 5.7–6.6 × 4.7–6.6 $\mu$ ; akinetes sphaerici, ut plurimum remoti a heterocystis, rarius eisdem contigui, diametientes 5.7–7.6 $\mu$ .

Typus lectus natans in vado aquae pluvialis in campo Instituti Indici Investigationis Agricolae, in urbe New Delhi, die 5 januarii anni 1957.

#### DISCUSSION

Fritsch (1949) basing his classification on the shape of the akinetes, divided all the species under three groups, viz., akinetes spherical or subspherical, ellipsoidal and cylindrical. Under 'spherical or subspherical', he further classifies the species according to the position of the akinetes, whether contiguous to or remote from the heterocysts. Regarding the position of the akinetes, Fritsch (1949) states, "most of the species, which normally form their akinetes from cells contiguous to heterocysts, only rarely depart from their habit. On the other hand, species forming series of akinetes from cells not adjoining the heterocysts, do occasionally form one next to a heterocyst." In the present form though usually the akinetes are remote from the heterocysts, it is not uncommon to find either a single or a chain of akinetes contiguous to the heterocysts and the author is convinced that every cell is potentially capable of transforming into an akinete (Venkataraman, 1957).

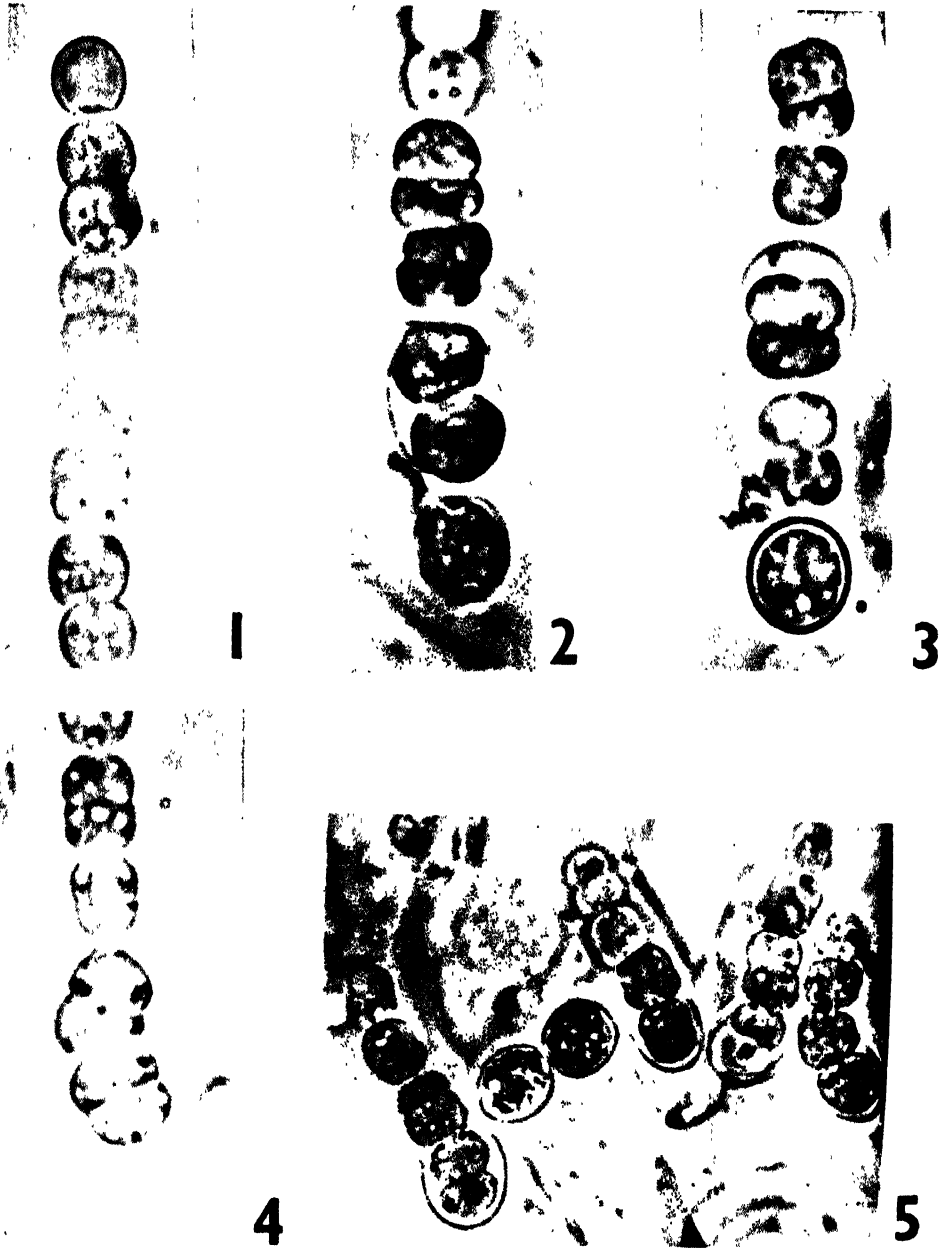
Since the formation of the akinetes remote from the heterocysts is the usual feature in the present form, it is thought advisable to place this alga along with the species which form spherical akinetes remote from the heterocysts like *A. fertilissima* and *A. gelatinicola*, although the present form is extremely variable in forming very frequently its akinetes contiguous to the heterocysts.

#### ACKNOWLEDGEMENT

The author records his sincere thanks to Dr. M. S. Randhawa for his continued interest and inspiration in the progress of this work. His grateful thanks are also due to Dr. B. P. Pal and Dr. S. M. Sikka for their keen interest and encouragement. His thanks are also due to Fr. H. Santapau for kindly providing the Latin diagnosis of this new form.

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Figs. 1-5. *Anabaena randhawae* sp. nov. Fig. 1. Trichome with a terminal heterocyst; Fig. 2-5. Stages in germination of akinetes; Fig. 2. Two-celled germling; Fig. 3. Three-celled germling; Fig. 4. Four-celled germling; Fig. 5. Four young germings with the broken akinete mother wall enclosing the basal portion of the germings; (Note in Figs. 2-4, the akinete wall enclosing the germling; Figs. 1-4.  $\times 2400$ ; Fig. 5.  $\times 1600$ ).



# CERTAIN RADIATION-INDUCED MORPHOLOGICAL ABNORMALITIES IN *CROTALARIA JUNCEA* L.

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## ABSTRACT

The  $X_1$ ,  $P_1$  and  $S_1$  progenies of *Crotalaria juncea* L. raised from the seeds exposed to X-rays, beta-rays from  $P^{32}$  and  $S^{35}$  have been studied.

A large number of morphological abnormalities like fasciation, lateral and base branching of the stems, splitting of leaves, sectorial chimaera and flowers with abnormal parts, etc., have been recorded.

The nature and significance of these abnormalities have been briefly discussed and it has been suggested that many of these abnormalities are non-genetical, arising as a result of physiological disbalance caused by the irradiation.

## INTRODUCTION

The importance of radiations in induced mutagenesis and crop improvement needs no emphasis. Various types of radiations like X-rays, beta-rays,  $\gamma$ -rays, ultraviolet rays and neutrons are now being used in a variety of crop plants all over the world and in many cases significant results have been obtained.

In *Crotalaria juncea* L., which is an important substitute fibre crop of India, the work was started with a similar view to isolate economic mutants with higher yield and/or better quality of the fibre.

The present paper deals with certain interesting morphological abnormalities, hitherto not recorded in this plant, obtained in the course of a preliminary dose trial experiment.

## MATERIAL AND METHODS

Seeds from a pure strain of *C. juncea* were exposed to the following radiation treatments at the atmospheric temperature and moisture conditions.

(i) X-rays—from a Philips Contact and Cavity Therapy apparatus working at 50 Kv and 2 mA, at three different doses, viz.,

(a) 5,000 r

(b) 10,000 r

(c) 15,000 r

(ii) Beta rays—from radioisotopes of Sulphur and Phosphorus (in aqueous medium) obtained through Messrs. Philips India (Private) Ltd., as follows :

(a)  $P^{32}$ —Initial activity 572.0  $\mu\text{c}$  for 2 durations, viz., 24 and 48 hours.

(b)  $S^{35}$ —At an initial activity of 909.0  $\mu\text{c}$  for 24 and 48 hours durations.

Seeds thus treated, were washed to remove the superficial isotope and were grown in pots as well as under field conditions and the data with regard to germination, mortality, height of plants, branching, flowering and yield, etc., were taken. However, in the present note, it is proposed only to deal with the various types of morphological abnormalities noted.

## MORPHOLOGICAL ABNORMALITIES AND DISCUSSION

During the course of these investigations, it was observed that there were a larger number of abnormalities in plants arising out of  $P^{32}$  treatments, fewer in X-ray and very few in  $S^{95}$  treated progenies, suggesting thereby that as compared to the other treatments,  $P^{32}$  produces more morphological abnormalities in *Crotalaria*.

(a) *Stem abnormalities :*

(i) The most frequent abnormality of the stem was the occurrence of "fasciation", resulting in a flattened or bilateral growth of the stem. Fig. 1 shows a characteristically fasciated stem of *C. juncea*. It was found that in cases of excessive fasciation, the stem tends to split up into two or three parts. Besides, in such plants, there is a clustering of the leaves near the apex. Generally, there is an increase in the number of leaves and the phyllotaxy is changed.

Such fasciations of stem and petioles following exposure to X-radiation have been recorded by Singh *et al.* (1939) in cotton and others in sunflower, tomato and cosmos. Beal (1949) recorded the phenomenon in species of *Digitalis* when treated with  $C^{14}$  and Sparrow and Singleton (1953) in *Xanthium* as a result of gamma radiations. White (1948) has reviewed the extensive literature on causes and occurrence of fasciation in plants both under natural and experimental conditions.

(ii) *Branching of the stem :* Lateral branching of the stem in the treated progenies, has also been found to be a character of irradiation. Jhonson (1936) in *Neurophila*, *Phlox*, *Helianthus*, *Lycopersicon*, Stanton and Sinclair (1951) in potato and Gunckel *et al.* (1953) recorded it in *Tradescantia*.

In the present investigations, it was found that in *C. juncea*, the induced branching is of two types :

(a) Excessive lateral branching, which although might be advantageous for the grain crops, is harmful in this crop as this type of branching breaks the fibre. This character was present both in X-irradiated and  $P^{32}$  treated progenies.

(b) Branching from the bottom is a desirable character in fibre yielding plants and was recorded only in the X-ray treated progenies. This increases the yield of fibre/plant, as each branch serves the purpose of a whole plant.

Fig. 2 represents a view of the treated progenies showing a plant with such branching. Fig. 3 depicts the unbranched stems of the 'control'.

(b) *Leaf abnormalities :*

As in the case of the stem, in the leaves also a number of morphological abnormalities were noted in the both X-ray and  $P^{32}$  treatments. The abnormalities recorded in course of present observations were :

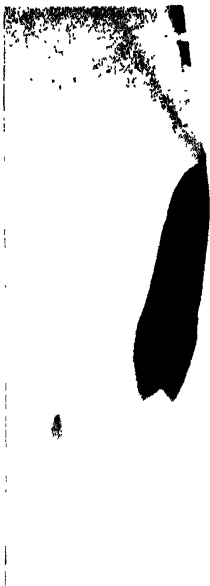
(i) *Crinkled Leaves :* In a single case in the population treated with 10,000 r of X-rays, a plant with crinkled and downy leaves (Fig. 4) was recorded. The leaves were smaller, more compact and of a darker shade, with a slightly twisted appearance. Fig. 5 shows the normal leaf character in this plant (control). Such crinkled leaves, following radiations have been recorded in *Lycopersicon* by Jhonson (1931) and others.

(ii) *Bifurcation of the leaves :* Bifurcation or forking of the leaves is yet another common response of plant to radiations. In course of present investigations, partially or completely bifurcated leaves were recorded in the  $P^{32}$  treated progenies. Fig. 6 shows the forking of the leaf tip, while Fig. 7 shows the whole lamina and a part of the petiole split into two.

Forking of the lamina and petiole is known to occur in nature in a number of plants, e.g., *Jasminum*, *Lonicera*, *Nyctanthes* and other genera of dicots (Bhatnagar, 1957). In *Crotalaria*, however, there is no previous record of leaf forking in nature.











Likewise, forking of leaves due to radiations has been reported by Jhonson (1926) in *Helianthus* and Haskins and Moore (1935) in X-irradiated plants of *Citrus*, resulting in the development of partially or completely bifoliate and trifoliate leaves which co-exist with the normal ones on the same plant. Gunckel *et al.* (1953) have, similarly, recorded dissected leaves in X-irradiated plants of *Tradescantia paludosa* and Sparrow and Singleton (1953), in *Xanthium* following chronic gamma exposure.

The observations suggest that the forked leaves arise as a result of physiological disturbances caused by irradiation, which in turn affect the development and differentiation of leaves and other parts and as such do not seem to be of much evolutionary significance.

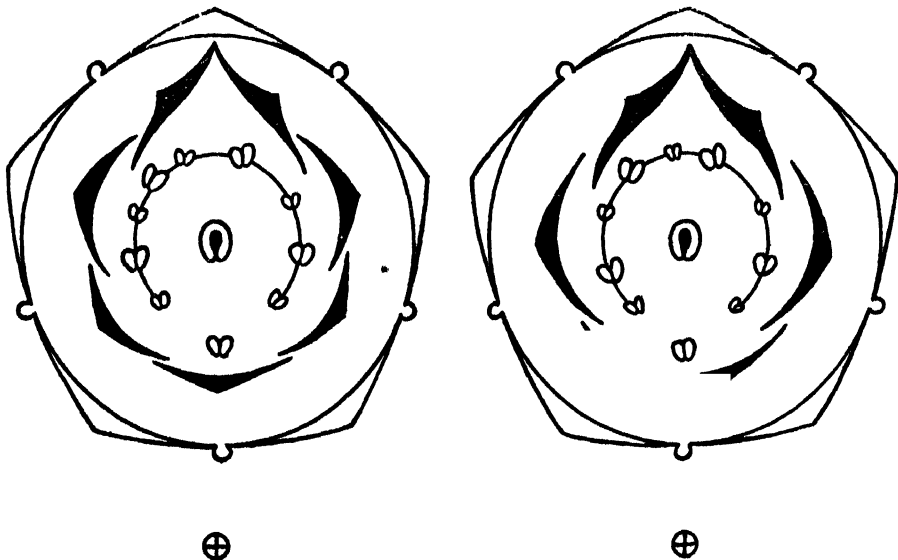
(iii) *Leaf chimaera* : Somatic mutations are known to be frequent in irradiated progenies. In the present case a small chimaeral twig bearing leaves was recorded in 10,000r X-ray treatment. One part of the leaves was normal green (Fig. 8). There is no previous record of naturally occurring chimaeras in *Crotalaria*. The present case resembles the condition met with in sectorial chimaeras.

(c) *Floral abnormalities* :

The golden yellow flowers of *Crotalaria juncea*, are the normal papilionaceous type, comprising of a large standard, two wings and a keel. There are 10 stamens and a monocarpellary ovary. The whole flower is enclosed by a single whorl of 5 gamosepalous calyx.

A number of floral abnormalities like the phylloidy of the floral parts, proliferation, adhesion and fasciation of the floral structures are, however, known to occur in nature in low frequency (Bose and Misra, 1938). But entirely different type of abnormalities were recorded in course of investigations on beta-irradiated progenies of this plant.

(i) *Abnormal floral parts* : In a single plant raised from  $P^{32}$  treated seeds, a flower was observed to possess three standards instead of the normal one (Fig. 9 and 10). The other parts of the flower were normal. Thus the organisation of the flower was altered as follows (Text-fig. 1) :

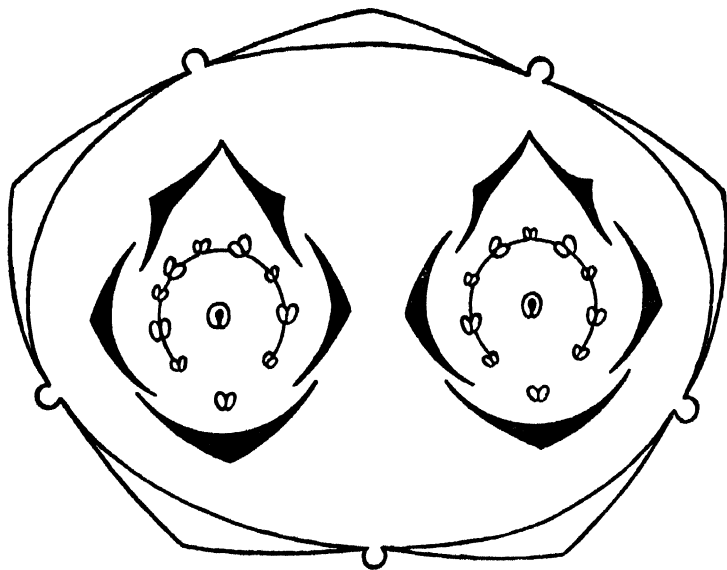


Text-fig. 1

Such an occurrence of extra one or two or the suppression of certain floral members has been reported to arise as a result radiation treatment by a number of workers. The most interesting examples are those in *Tradescantia* (Gunckel *et al.*, 1953 and Sparrow and Singleton, 1953).

(ii) *Double flower* : In the same plant still another interesting abnormality was the occurrence of two complete flowers enclosed in a single whorl of calyx. Fig. 11 is the photograph showing the double flower on a normal pedicel, while Fig. 12 shows the double nature of the ovary in the same.

The detailed floral structure may therefore, be shown as follows (Text-fig. 2) :



Text-fig. 2

According to Jhonson (1926), who recorded a large number of abnormalities in X-irradiated *Helianthus*, fasciation of the floral axis is responsible for the formation of extra members in a flower or double flowers. But the present view is that this could be explained on the basis of excessive proliferation of the plant parts arising due to physiological disturbance caused by irradiation.

(iii) *Suppression of the flower and rupture of the keel* : In certain cases in the  $P^{32}$  treated progeny, the flower-buds were found to develop normally, which did not open in the end. In such cases, the keel which normally persists till long after fertilisation, was found to rupture with the stigma protruding out. However, no fruits were formed in such flowers.

(iv) *Change in the colour of standard* : The  $S^{32}$  treated progenies were marked by the absence of the gross morphological abnormalities enumerated earlier. However, in some plants of this treatment, it was noted that the colour of the flowers had changed appreciably over the control. In all these cases, the dorsal surface of the standard developed a bright crimson colour. These plants maintained this character till the end and no normal coloured flowers were found to occur on these plants.





Such changes in the colour of the flowers, etc., unlike other morphological abnormalities may sometimes be genetical in nature. Lawrence and Struggess (1957) in a recent paper, have advanced a hypothesis explaining the evolution of flower colour in *Streptocarpus* through the successive mutation of genes, their becoming dominant and finally epistatic to their predecessors. If this hypothesis is correct, the variations in the flower colours of certain irradiated plants may be explained on the basis of mutation of the gene or genes controlling the expression of pigment, as a result of this treatment. However, some of the radiation-induced colour variations are supposed to be chimaeral in nature, e.g., the red colour induced in carnation (*Dianthus caryophyllus*) by the X-rays (Sagawa and Mehlqvist, 1956), while till others are believed to be arising as a result of mutations or deletions, e.g. in *Antirrhinum majus* through chronic gamma irradiation (Sparrow and Pond, 1956). Similar change of colour in flowers has also been reported by other workers.

The occurrence of abnormal flowers side by side with normal ones on the same plant, together with the fact that most of these abnormalities are not passed on to the subsequent generations, has led Nickson (1952) and Gunckel *et al.* (1953) to suggest that the physiological disturbances caused by irradiations are responsible for the morphological abnormalities described. These physiological effects may be caused by the damage or inactivation of the enzymes, substrates, hormones or physico-chemical changes in the permeability and viscosity of the cytoplasm, etc. It also seems that as the dose of irradiation is not so high as to destroy the vital substances, the plant continues its life-cycle with certain abnormal features. Physiological action of the radiation on the cytoplasm has also been held responsible for the histological changes in the stems and petioles of the irradiated progenies of plant (Beal, 1949).

The investigations on the anatomical and cytological aspects of these abnormalities are in progress, while the study of inheritance of these characters in subsequent generations is also being taken up.

#### ACKNOWLEDGEMENT

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\* Not seen in original.

# GROWTH AND DEVELOPMENT OF SEPTATE AND CRYSTALLIFEROUS FIBRES IN SOME INDIAN TREES

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(Communicated by K. A. Chowdhury, F.N.I.)

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## ABSTRACT

1. The development of septa in the wood fibres of two hardwoods, one ring-porous, *Lagerstroemia speciosa* (L.) Pers. and another diffuse-porous, *Protium serratum* (Wall. ex Colebr.) Engl. is reported.

2. Septa are formed in the fibres, as a result of the division of the protoplast which consists of true Karyokinesis followed by Cytokinesis at right angles to the long axis of the fibres, after the elongation of the cells is completed and secondary walls are laid in them.

3. The oldest fibre formed in a season, becomes septate first and the younger ones gradually become septate in centrifugal manner. The development of septa apparently takes place soon after the fibres attain maturity, as the fibres which fail to develop septa at the time remain non-septate.

4. Some correlation has been noticed between the time required for the fibres to attain maturity and the growth period. In *L. speciosa* in which the growth period is short, the septa are developed within a short time compared to *P. serratum*, which has a longer period of growth.

5. The number of septa formed in a fibre has been found to be more variable in *L. speciosa* than in *P. serratum*. The variability of this character and its reliability as a diagnostic feature in wood is discussed.

6. Crystalliferous fibres are formed in *L. speciosa* at the end of the growing season. Some of the septate fibres show nuclear division once again at that time. Each compartment divides into several locules and in each of them a crystal develops in the cytoplasm. The importance of crystalliferous fibres in the identification of woods is also discussed.

## INTRODUCTION

Septate fibres are of common occurrence in the secondary xylem of many dicotyledonous families. They are seldom found throughout a family ; even in a single genus all the species may not always show septate fibres (Metcalf and Chalk, 1950 ; Spackman and Swamy 1949). Furthermore, the frequency and distribution of these fibres are known to vary from tree to tree and in some cases in different parts of the same tree (Chowdhury, 1954).

Standard text books on plant anatomy give rather scanty information on septate fibres and their importance in systematic classification of secondary xylem. Credit for throwing some light on this type of fibres goes to Vestal and Vestal (1940). They studied only one species, namely *Hypericum androsaemum* L. but by oversight did not give the ontogeny in detail. The present investigation was, therefore, taken up to find out its course of development from inception to maturity. In order to find out how the frequency of septate fibre varies in some species while in others it remains constant, two such types were selected for study, namely, *Lagerstroemia speciosa* (L.) Pers. and *Protium serratum* (Wall. ex Colebr.) Engl. These also represent the two main groups of dicotyledonous woods ; the former belongs to the ring-porous group, while the latter to the diffuse-porous. Some preliminary notes have already been published on this investigation (Purkayastha 1953, Chowdhury *et al.* 1956). The present paper deals with the detailed information of the different stages of development of septate fibre up to its maturity. While studying *L. speciosa* (L.) Pers. it has been noticed that some septate fibres turn



crystalliferous at the end of the growing season. Complete information on the formation of these crystalliferous fibres has also been included in the paper.

### MATERIAL AND METHODS

Material examined consisted of both elongating current year's shoots and mature one to two year old branches. For each species five trees growing in New Forest, Dehra Dun, were selected, and their growth data recorded. The material was collected at weekly intervals till the fibres became septate and later on at fortnightly intervals till the cessation of growth in November. The older shoots were collected only after the commencement of radial growth. All material was fixed in Formalin-Acetic-Alcohol at a time when the nuclear division associated with the formation of septa was most active. The best time for the taking out material, which was determined after several trials, was found to be different for the two species. In *L. speciosa* the best time was found to be between 6.30 and 7.30 a.m. while in *P. serratum* between 5 p.m. and 6 p.m.

The study was mainly based on a large number of longitudinal sections cut on a sliding microtome and stained in Heidenhein's Haematoxylin and saffranin. The results obtained during the first year of study were confirmed by observations made during the second year. There was little difference between the materials collected in two years.

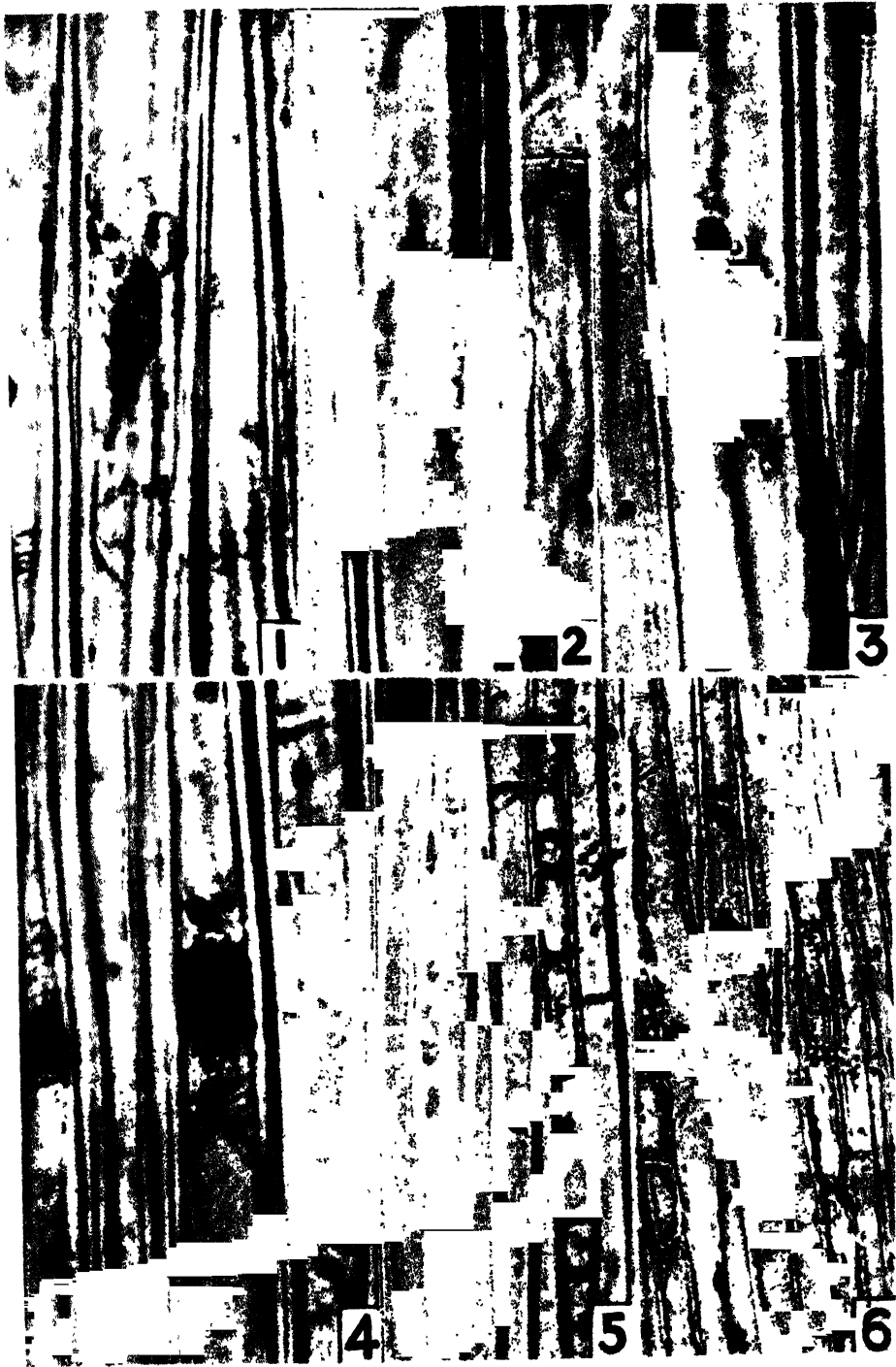
### RESULTS

#### I. *Lagerstroemia speciosa* (L.) Pers.

The trees at Dehra Dun are generally leafless during February and March. In the latter part of March, new buds start coming out from the axils of the leaves, as indicated by scars. These start growing into new shoots by the end of March. The extension growth of these shoots is generally completed in ten to fifteen days. The flowering shoots also come out about the same time. A second flush of extension growth takes place usually in the beginning of July and continues also for about a fortnight. The second extension growth is rather irregular in this species, some branches not having it at all. In most of the branches after completion of the first height growth, the terminal buds drop off. In many of them, however, new buds developing from the axils of the terminal leaves take the place of terminal buds and start growing again in July. At the same time, new buds can also be seen developing on the older branches, which have not grown during the first height growth. This type of irregular growth of shoots from older branches has been observed from the beginning of July till October. Occasional development of flowering shoots has been noticed even in December. Leaves are shed in January or early February. The radial growth in the older branches has been observed to take place long after the completion of the first extension growth. The cambium in these branches remains inactive till late July or the early part of August.

#### *Formation of septate fibres in the elongating shoots.*

The septa are formed about two weeks after the fibres are cut off from the cambium. By this time the elongation is completed and the secondary walls of the fibres are fully laid down. The first fibres to become septate are those in the region nearest to the protoxylem vessels at the base of the shoots. From here the formation of septa gradually spreads outwards towards the cambium and also upwards towards the tip of the shoot.



Figs. 1-3, *Lagerstroemia speciosa* (L.) Pers. Stages in the formation of septa; 1, Metaphase 2, early telophase; 3, late telophase. Fig. 4, *Protium serratum* (Wall. ex Colebr.) Engl. radial section showing progressive development of septa from inside (right) to outside (left) ( $\times 1500$ ). Figs. 5 and 6, stages in the development of crystalliferous fibres; 5, division of the nucleus in one of the compartments of a septate fibre; 6, simultaneous division of nuclei of different compartments of a septate fibre ( $\times 450$ ).



The formation of septa in the fibres is by nuclear division followed by cytokinesis at right angles to fibre walls. All fibres before the formation of septa, are characterised by an oval elongated nucleus, with a single nucleolus. The nucleus passes through the usual changes during division. The spindle figure becomes apparent at the close of the *prophase*. In *metaphase*, the spindle is generally seen at an angle to the long axis of the fibre (Fig. 1). In *telophase*, the phragmoplast begins to extend laterally in the equatorial region till it touches the walls of the fibre (Figs. 2 and 3). The cell plate is formed in the equatorial region which divides the fibre lumen. The daughter nuclei pass into the resting stage after migrating to the centre of the newly formed compartments. The septum finally develops in the region of the cell plate and in staining behaviour resembles middle lamella. In the mature wood the septa appear to be composed mostly of lignin. They are completely dissolved by repeated chlorination and subsequent treatment with dilute ammonia but they are insoluble in 72 per cent sulphuric acid.

Although formation of septa is evidently correlated with cell maturation, it has been noticed that in the newly formed shoots, nuclei of a few fibres fail to divide on attaining maturity, and as a result, these fibres remain non-septate. Apparently these nuclei do not divide later.

#### *Formation of septate fibres in the older branches.*

The septate fibres are formed in a similar manner about a fortnight after they are cut off from the cambium. The newly formed fibres adjacent to previous year's wood become septate first and gradually the fibres nearer cambium become septate. The septate fibres in the elongating shoots, however, differ from those in the older branches as regards the number of septa they contain. In the elongating shoots the septate fibres are generally partitioned by a single septum, whereas those in the older stems are usually divided by three septa, although fibres with one or rarely two septa, are sometimes met with. These septa are formed by a second division of the daughter nuclei after the formation of the first septum. The occasional presence of fibres having one or two septa is due to the failure of both or one of the daughter nuclei to divide again.

#### *Formation of crystalliferous fibres.*

After the formation of septa, the nuclei in the fibres remain in 'resting stage' for a long time. The nuclei are visible in these fibres till heavy deposition of starch occurs in them. The starch deposition takes place twice in a year, once in June in the shoots developed in April and again in November in the later formed tissues. Along with the deposition of starch some of the septate fibres, particularly those contiguous to longitudinal parenchyma and rays, also become crystalliferous. These fibres are then subdivided into small locules in which solitary crystals develop.

The first stage in the formation of crystalliferous fibres is the renewed mitotic divisions in the septate fibres. The fibres, which ultimately become crystalliferous, are distinguished from the others by their enlarged nuclei and deeply stained protoplasts. The stages in the division are similar to those already described except that the daughter nuclei divide several times, successively and simultaneously, and the fibre is subdivided into small locules within a short time (Figs. 5 and 6).

Each of the locules thus formed, contains a round nucleus in the centre surrounded by a thick mass of cytoplasm without any vacuoles. The crystal first appears as a small cytoplasmic inclusion, which is an exact replica of the fully grown crystal in shape. As the crystal grows, it pushes the cytoplasm and the nucleus to the periphery and ultimately the cytoplasm forms just a lining round the crystal. The nucleus, however, remains prominent throughout the development of the crystal.

Generally all nuclei in a fibre divide and the entire fibre becomes crystalliferous. But nuclei of all the compartments of a septate fibre always may not take part in the formation of these locules which ultimately become crystalliferous. Occasionally only half of them divide to form the crystalliferous locules while the other half remains unchanged.

## II. *Protium serratum* (Wall. ex Colebr.) Engl.

In this evergreen species also two distinct periods of extension growth have been observed. However, the extension growth in this species differs from that of *L. speciosa* in some respects. All the branches in *P. serratum* grow during both the periods of extension growth and the terminal buds do not fall off after the height growths are over. The first extension growth starts in the beginning of March and continues for about 3-4 weeks. By the middle of April generally all the leaves of the new shoots are fully mature. It is about this time, that the previous year's leaves turn yellow and start falling off. The second extension growth generally starts by the middle of June and also continues for about a month.

As in *L. speciosa* no activity of the cambium of the older branches has been noticed during the first extension growth. The diameter growth in these parts usually starts by the end of June or the first week of July.

The development of septa in the fibres takes place first in the oldest fibre formed in a season and then gradually in the younger ones, as in *L. speciosa*. In the elongating shoots, the formation of septa is noticed first in the fibres nearest to the protoxylem vessels, when the shoots are about four weeks old. In the older branches also the septa are developed in the fibres, adjacent to the previous year's wood, about a month after the initiation of radial growth. The stages in the formation of septa are similar to those of *L. speciosa* (Fig. 4). However, both in elongating shoots, as well as in older branches, the nuclei of the fibres, after attaining maturity, divide several times and consequently a varying number of septa are formed in the fibres. Moreover, as the fibres mature, the nuclei of all the fibres divide with the result no fibres remain non-septate. The septa, although similar to *L. speciosa*, are more resistant to the chemical action.

As in *L. speciosa*, the nuclei in the fibres are visible throughout the year till the visibility is obscured by heavy deposition of starch, which appear to be correlated with the slackening of growth. Unlike *L. speciosa*, however, the nuclei in these fibres do not show any division again at this time and no crystalliferous fibres are formed.

## DISCUSSION

Considerable work has been done by Chowdhury and Tandan (1950) on the growth of broad-leaved trees of India. They have found that extension growth usually precedes radial growth by about 2 weeks to 3 months, in both ring-porous and diffuse-porous species. Their observations differ considerably from those reported from Europe and North America where both the height and the diameter growth start simultaneously. The difference observed between the time of initiation of extension and radial growth in both these species confirms Chowdhury and Tandan's findings.

The stages in the division of the protoplast associated with the formation of septa are similar to those described by Vestal and Vestal. However, in the two species investigated, there are some differences as regards the time required for the development of septa and in the number of septa formed in a fibre. These are tabulated below :—

| Species            | Time required for the development of septa in the fibres | Number of septa formed per fibre     |                           | Nature of deposits in the fibres                     |
|--------------------|--|--------------------------------------|---------------------------|--|
|                    |  | In elongating shoots                 | In older branches         |  |
| <i>L. speciosa</i> | about 2 weeks  | 1, a few also remaining non-septate. | 3, but occasionally less. | Starch but a few fibres also become crystalliferous. |
| <i>P. serratum</i> | about 4 weeks  | 3 or more.                           | 3 or more.                | Starch, but no crystals.                             |

Since septa are developed after secondary walls are laid down, their formation may be taken as an indication of the maturity of the fibres. The development of septa in the fibres from the oldest to the youngest in a centrifugal manner also suggests that the formation of septum takes place immediately after the fibres attain maturity. It is interesting to note in this connection the apparent correlation between the growth period and maturity of the fibres. In the ring-porous *L. speciosa*, the septa are developed when the fibres are about two weeks old and the extension growth is also completed within this period. Whereas in the diffuse-porous *P. serratum* the septa are developed after about 4 weeks and the extension growth also continues for about a month. The occurrence of fewer number of septa and the occasional presence of non-septate fibres in the elongating shoots of *L. speciosa*, probably indicates the comparatively variable nature of this character in this species. A preliminary survey of the septate fibres in the wood of some families like *Anacardiaceae*, *Burseraceae*, *Combretaceae*, *Euphorbiaceae*, *Meliaceae* and *Lauraceae* has also shown that although the presence of septate fibres is generally a constant feature for a species, in some of the woods the percentage of septate fibres and the number of septa per fibre may vary in a similar manner. More observations on this point are necessary to bring out the true significance of this character as a diagnostic feature.

The nuclear divisions which precede the formation of crystalliferous locules are similar to those of the formation of septa in the fibres. However, the nuclei of different compartments of a fibre, divide several times and these divisions take place simultaneously. Similar simultaneous division of the nuclei in the phloem fibres of tobacco has been reported by Esau (1938). In these phloem fibres, however, the karyokinesis is not followed by cytokinesis which results in multinucleate condition. In the formation of crystalliferous locules, on the other hand, normal division of the protoplast occurs and septa are formed in the region of the cell plate during cytokinesis.

It is generally believed that Calcium oxalate is an excreted waste product of plant metabolism. Milanez, quoted by Chattaway (1937), has suggested that the frequent occurrence of crystals in the terminal parenchyma (as in many of the *Caesalpinaceae*) may be the result of the accumulating of waste products from the activity of the entire growing season. The development of crystals, both in the wood fibres as well as in other woody tissues, at the end of the growing season lends support to the view expressed by Milanez. Chattaway, however, thinks that although this is a very probable explanation of the crystals in the chambered parenchyma, it does not seem equally applicable to the solitary crystals that are often found scattered irregularly in rays and parenchyma.

A survey of the literature reveals that crystalliferous fibres have been grouped under two classes by some workers (Metcalf and Chalk, 1950; Pearson and Brown 1932). Some septate fibres show only a few septa and only some of these chambers formed thereby contain crystals. On the other hand, some such fibres are subdivided into small locules and in each of these locules or chamber crystals are

formed as in *L. speciosa*. The value of the latter type of fibres for generic classification of wood appears to be good, but needs further confirmation.

In this connection, the difficulty of detection of crystalliferous parenchyma and crystalliferous fibres must be pointed out here. Chattaway (1937) while working on *Sterculiaceae* reported presence of crystalliferous fibres in *Sterculia*. Milanez (1937, 1939), however, questioned it because he was of the opinion that these crystal cells in *Sterculia* are parenchymatous in origin and not prosenchymatous. This was later confirmed by Chattaway (1939) herself. All these mean that one has to be very careful in describing the presence of crystalliferous fibres and crystalliferous parenchyma. A mere examination of sections depicting different views of a block of wood may lead to confusion. The final classification of these crystalliferous elements will rest on the examination of the macerated material.

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PHYSIOLOGICAL ADAPTATIONS OF DUSKY COTTON BUG,  
*OXYCARENUS HYALINIPENNIS* (COSTA) (HETEROPTERA ;  
LYGAEIDAE) TO ITS HOST PLANT, COTTON

PT. I. DIGESTIVE ENZYMES IN RELATION TO TISSUE PREFERENCES

by K. N. SAXENA and PREMLATA BHATNAGAR, *Department of Zoology,  
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ABSTRACT

Present study was undertaken to ascertain correlation between relative preference of *Oxycarenus hyalinipennis* (Costa) for different parts of cotton plant and its ability to utilise the nutrients available in these parts.

The insect shows maximum preference to cotton leaf as the source of food and least to cotton-seed. While feeding on leaf they draw food-sap mostly from mesophyll and phloem tissues, rarely from xylem vessels and never from the oil-glands scattered all over the leaf and other parts of the plant.

The insects feeding on cottonseed draw food material from outer or inner integument of the seed coat. They never penetrate their stylets deeper than the seed coat and, consequently, never feed on the kernel or embryo of the seed.

Nutrients available to the insects feeding on cotton leaf are proteins, some free amino acids, starch, sugars (sucrose, glucose and fructose), and fats. Those available in the cottonseed coat are pentosans, sugars (raffinose, sucrose, glucose and fructose), free amino acids, slight quantities of proteins and fats.

Study of the distribution of digestive enzymes indicates that cellulase, hemicellulases, inulase,  $\beta$ -glucosidases,  $\beta$ -galactosidases, polypeptidase, and lipase are absent. Amylase, maltase, invertase,  $\alpha$ -galactosidase, proteinase and esterase occur in the salivary secretion and in the midgut. Their occurrence in the hindgut is quite irregular. Trehalase has been detected only in the midgut. Maximum activity of almost all these enzymes occurs at pH 5.4. The invertase, present in the digestive tract of *Oxycarenus*, is a glucosaccharase.

Correlation between the nutrients available in cotton leaf or in seed coat and ability of the insect to utilise the nutrients has been discussed. Cotton leaf appears to be a much better source of food for *Oxycarenus* than cottonseed.

INTRODUCTION

Genus *Oxycarenus* Fieber includes a number of species which are known to be pests of cotton plant in different parts of the world. Their biology has been studied by various workers and there has been some disagreement regarding the site of feeding, and nature of injury caused by these insects. Most workers consider that *Oxycarenus* feeds mainly on the seeds of cotton and causes severe damage to their tissues (Peacock, 1913 ; Balls, 1915 ; Misra, 1921 ; Kirkpatrick, 1923). Kirkpatrick (1923) further believes that this insect visits green leaves or epicalyces etc. only to imbibe moisture from the glands present on those parts of the plant. According to some other workers *Oxycarenus* feeds on the blossoms (Sickenberger, 1890), squares, buds and young bolls of cotton (Willcocks, 1906 ; Adair, 1918 ; Misra, 1921) in addition to the seeds. Investigations of Saxena and Krishna (1958) on the orientation and site of feeding of *O. hyalinipennis* on cotton plant indicate that this insect, when offered a choice from amongst cotton leaves, epicalyces, green bolls, opened bolls, seeds, lint, flowers and flower buds, prefers to feed mostly on green leaves or epicalyces. Next in order of preference come



other green parts of the plant, and seeds come the last. In fact seeds are fed upon much less frequently than the leaves.

Little is yet known whether *Oxycarenus* is better adapted to utilise nutrients available in the cottonseed than those in the leaf or vice versa. Present work has been undertaken in order to investigate this aspect.

### MATERIAL AND METHODS

Adults of *Oxycarenus hyalinipennis* (Costa)\* were used for the present work. For all the experiments reported in this paper freshly collected insects were allowed to feed on distilled water for 48-72 hours in order to clear their alimentary canal by flushing.

In order to determine the plant tissue from which the food-sap is drawn, the insects were given access to different parts of cotton plant and allowed to feed on them. Since cottonseed has been reported by Kirkpatrick (1923) to be the chief site of feeding and leaf to be the main source of food by Saxena and Krishna (1958), only these two parts were selected for the present study. Insects to be fed on leaves were provided with short twigs of cotton plants bases of which were kept immersed in water to prevent wilting. The seeds supplied to the insects were freshly collected from fresh or old open cotton bolls and were delinted as completely as possible. Some fuzzy hairs were, however, left behind on the seed-surface. The seeds from freshly opened bolls had a fine film of moisture on their surfaces due to moist lint surrounding them. The seeds from the old opened bolls had, on the other hand, dry surface. It was difficult to make the insects feed on cottonseed. However, they could be made to do so with a little less difficulty by applying a fine film of moisture to the surface of the seed. This was done by giving the delinted seed a dip in water and then wiping its surface with filter paper, drying the fuzzy hairs as completely as possible. Whatever trace of moisture was left on the surface of the seed was enough to induce the insects to feed on it. It may be noted that no water could diffuse into the interior of the seed by this treatment nor was there sufficient moisture for the insects to drink it from the surface and prevent them from penetrating their stylets deeper into the seed tissues.

Whenever an insect penetrated its stylets into the leaf or seed tissue it was allowed to feed for 1-2 minutes and then its beak was clipped off by means of a pair of very fine scissors. In the case of insects feeding on cotton leaf the stylets *in situ* with the leaf were fixed in Formol-acetic acid-50 per cent alcohol mixture (5 : 5 : 90 v/v) for about 12 hours. Freehand sections of the fixed material were cut, stained in Safranin, counterstained in Fast Green and finally mounted in canada balsam. In case of insects feeding on cottonseed, the stylets *in situ* with the seed were treated with 10% KOH solution for 4-7 days in order to soften the seedcoat. Fairly thick sections of these seeds were then cut, mounted in glycerine and examined in reflected light under a microscope. Various parts of the seed, including the two distinct outer and inner integuments of the seedcoat, could be differentiated without difficulty.

In order to ascertain the nutrients that may be available to the insects feeding on cottonseed and leaf, a knowledge of chemical composition of these parts is essential. Some information on chemical composition of the entire cotton leaf and different parts of cottonseed is already available and may be made use of in this paper. However, additional information on the free amino acids and sugars present in the cotton leaf and seedcoat (hull) was desirable and was obtained in the present work.

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\* The insects were kindly identified by Dr. Reece I. Sailer, U. S. Dep. Agric. Entomological Research Bureau, Washington, D.C., for our colleague Dr. M. K. Dutt.

Cottonseed hulls from 100 freshly collected seeds and 50 fresh leaves were separately homogenised in 80 per cent ethanol. The leaves used for extraction were collected both at night as well as during the day and were transferred to 80 per cent ethanol immediately after plucking. The homogenate was centrifuged and the supernatant was concentrated to a volume of 5 ml. by evaporation in a stream of air. 0.05 ml. samples of the concentrated extract were applied on 18" by 18" sheets of filter paper (Whatman No. 1) and subjected to paper chromatography by the descending technique. For sugars single dimensional chromatograms were run using the solvent system *n*-butanol—acetic acid—water (4:1:5 v/v). For amino acids two dimensional chromatograms were run employing the above solvent for the first run and phenol-water (8:2 w/w), containing 0.04 per cent 8-hydroxyquinoline, as the second solvent. Sugars on the chromatograms were revealed as brown or yellowish brown spots after spraying with benzidine-trichloroacetic-acid reagent (Bacon and Edelman, 1951) and heating at 105°C for about 10 minutes. Amino acids were revealed by spraying with 0.25 per cent solution of ninhydrin in acetone and heating at 60°C for about 10 minutes.

To determine the distribution of enzymes in different parts of the digestive tract of *Oxycaenus*, the insects fed on water for 48–72 hours were starved for 3–4 hours under 0% R.H. They were then made to drink water. An hour later they were dissected one by one and aqueous extracts of their salivary glands, first, second, and third ventriculi, and the hindgut were prepared according to the method described by Saxena (1954). For each enzymatic determination extracts from 10–12 individuals were pooled together, centrifuged and the volume of the supernatant was made up to 1 ml. with distilled water. The clear extract was divided into five equal lots of 0.2 ml. each, arranged in two series A and B. The former contained three lots while series B contained two lots. The extracts of series B were heated in boiling water bath for about an hour and served as controls. To each of the extracts of both the series were added 0.1 ml. of a suitable substrate solution or suspension and 0.2 ml. of a suitable citrate-phosphate buffer. In the absence of any information on the pH optima of the digestive enzymes of *Oxycaenus*, the three extracts of series A were buffered to three different values of pH 3.0, 5.4, 7.2, selected arbitrarily. The reaction of the two extracts of series B was adjusted to pH 3.0 and 5.4 respectively. The extract-substrate-buffer mixtures were incubated at 37°C for about 12–24 hours and the presence or absence of the substrate or products of its hydrolysis was chemically determined as described below.

Names of enzymes investigated and the substrates employed for their detection are given in Table 1. For the detection of amylase, 0.03 ml. samples of all the five incubated mixtures were placed 1" apart on a strip of filter paper. After drying, the strip was given a dip in 1.0 per cent ethanolic solution of iodine. Incubated mixtures containing undigested starch would show up as blue spots on the paper strip, the intensities of the spots depending on the amount of starch present. In case of complete digestion of starch no blue colour would develop. Visual comparison of the intensities of blue colour gave an approximate idea about the relative activity of amylase in extracts of different regions of the gut under different conditions of hydrogen ion concentration.

For detection of the rest of the carbohydrases, presence or absence of hydrolytic products of the substrates in incubated mixtures was determined by paper partition chromatography. 0.03 ml. samples of incubated mixtures were placed 1" apart along one of the longer edges of 22" by 18" sheet of filter paper (Whatman No. 1). Suitable reference sugars were also applied on the same paper-sheet. The solvent *n*-butanol-acetic acid-water (4:1:5 v/v) was allowed to run by descending technique for 48 hours. The chromatogram, after drying, was developed with benzidine-trichloroacetic acid reagent as described before. Sugars in the incubated mixtures, which showed up as brown or yellowish brown spots on the chromatogram,

were identified by comparing their positions on the chromatogram with those of the reference sugars. Appearance of sugars other than the substrate in any incubated mixture would indicate hydrolysis of the substrate due to the activity of the corresponding enzyme. Visual comparison of the intensities of spots of undigested substrates would give a rough idea about the degree of enzymatic activity at three different pH values under consideration.

For proteinases a different procedure was adopted. As stated before, 1 ml., of the original extract of each region of the digestive tract was divided into five lots of 0.2 ml. each, arranged in two series A and B, the latter serving as control. All of these were buffered as in the previous cases but no substrates were added to the extracts. On the other hand, 0.1 ml. samples of each of the five lots of buffered extracts were placed, side by side, on the gelatinised surface of a 5" by 1.25" strip of a photographic plate. The extracts on the strip were incubated in a moist chamber for about 12-14 hours and then the strip was washed in cold distilled water. After drying in air it was immersed in a bath of 0.1 per cent ethanolic solution of bromophenol blue for about 5 minutes. After drying it again, the strip was washed in a bath of 1 per cent acetic acid. The gelatin of the photographic plate, being a protein, would take up a deep blue stain which would persist even after acidifying with acetic acid. Hydrolytic products of proteins such as

TABLE I

*List of the enzymes tested for and the substrates employed*

| Name of Enzyme           | Name of substrate employed  |
|--------------------------|---|
| <b>CARBOHYDRASES :</b>   |   |
| Amylase                  | Soluble starch, 0.3% aqueous solution containing a little sodium chloride.  |
| Inulase                  | Inulin, 1% suspension in water.   |
| Cellulase                | Cellulose powder, 1% suspension in water.   |
| Hemicellulases           | a. Pentosans extracted from cottonseed hull according to the method of Jernyn (1955), 1% suspension in water.<br>b. Gum arabic, 1% aqueous solution<br>c. Agar agar, 1% aqueous solution. |
| Maltase                  | Maltose, 5% solution.   |
| $\beta$ -glucosidases    | a. Cellobiose, 5% solution.<br>b. Salicin, 5% solution.   |
| $\alpha$ -galactosidases | a. Raffinose, 5% solution.<br>b. Melibiose, 5% solution.  |
| $\beta$ -galactosidase   | Lactose, 5% solution.   |
| Invertases :             |   |
| B-h-fructofuranosidase   | a. Sucrose, 5% solution.<br>b. Raffinose, 5% solution.  |
| Glucosaccharase          | a. Sucrose, 5% solution<br>b. Melezitose, 5% solution   |
| Trehalase                | Trehalose, 5% solution  |
| <b>PROTEINASES :</b>     |   |
| <b>POLYPEPTIDASES :</b>  |   |
| <b>ESTERASES :</b>       |   |
| Lipase                   | Olive oil emulsion, prepared as described by Baldwin and Bell (1955)  |
| Esterase                 | Ethyl butyrate.   |

polypeptides and amino acids do not give this test since their blue stain changes to yellow on acidifying (vide Fiegl, 1954, for explanation). Presence of proteolytic

enzymes in any of the incubated mixtures would bring about digestion of gelatin at the spot where the incubated mixture was placed on the photographic plate. As a result of this the blue colour at that particular spot would fade out. The degree of fading of blue colour would depend on the enzymatic activity and in the case of complete digestion of gelatin the spot might become transparent. Visual comparison of the intensities of blue colour developed by different incubated mixtures would give a rough idea about the degree of proteolytic activity in different parts of the gut and under different conditions of hydrogen ion concentration.

For the detection of lipase the method described by Baldwin and Bell (1955) was employed. Esterases acting on lower esters were also tested in the same way, with emulsion of ethyl butyrate as the substrate.

#### TISSUE PREFERENCES

As mentioned before, *Orycarenus hyalinipennis* has been reported to feed mainly on cottonseeds by some workers, particularly by Kirkpatrick (1923). On the other hand, Saxena and Krishna (1958) observed that this insect prefers to feed on green parts of the cotton plant, particularly on leaves. Since each of these parts is made up of a number of different tissues which differ in their structure and chemical composition, the nutrients available to *Orycarenus* in any one part of the plant will depend upon the tissue from which the sap is drawn. It was, therefore, considered necessary to determine the exact tissue of cottonseed or of leaf from which the insect draws its food. Leaf was taken to represent the green parts of the cotton plant since it is shown maximum preference by the pest.

*Preference for Leaf-Tissues.* Both the adults as well as the nymphs feed mostly on the lamina of cotton leaf, and occasionally they also feed on leaf-petiole. The insects feeding on the lamina may attack it from any of the two surfaces, preferably the one in shade. The point at which the stylets are introduced into the leaf-tissue is selected quite carefully. The insect always avoids the so-called 'oil-glands' which are profusely distributed all over the cotton plant (Brown, 1938). The stylets are pierced through the surface of the leaf in-between the oil-glands. In most cases the point of entry lies in the angles of veins or veinlets; but occasionally it may lie in the area in-between the veins or directly on the vein or veinlet. As the stylets penetrate into the tissues intracellularly the cells are torn off.

The tissue up to which the stylets extend and from which the sap is drawn out may vary with different individuals and with the same individual. Stylets of insects feeding on the lamina extend mostly into the mesophyll tissue; both the palisade layer of cells as well as the spongy parenchymatous tissues are equally preferred. In the case of insects feeding directly on the vein or veinlet the stylets usually extend up to the phloem tissue also. Both the phloem parenchyma and sieve tubes may be tapped. The insects which feed on leaf-petiole introduce their stylets mostly into the parenchymatous tissue but often they are found to tap the phloem tissue as well. Rarely the stylets have been noticed to extend up to xylem vessels. There is no indication that the introduction of the stylets into one or the other tissue is limited by the length of the stylets or of the labium. On the same leaf some individuals may extend the stylets up to the phloem tissue and others may stop short at the parenchymatous tissue.

These observations make it clear that so far as leaf as the source of food is concerned, *Orycarenus* draws food sap from almost all parts of the cotton leaf, except from the oil-glands. However, the insect shows a little greater preference to mesophyll tissue and phloem parenchyma than to phloem vessels.

*Preference for Seed-Tissues.* The insects have been observed feeding on cottonseed much less readily than on leaf. Presence of a fine film of moisture on the surface of the seed is fairly helpful in making the insects feed on the seed.

Still, hardly 8-10 insects out of a batch of 50 feed on the cottonseed in about 6-8 hours. Most of them probe their beaks here and there on the surface of the seed and then move away.

Insects feeding on cottonseed do not seem to show any choice for any particular spot on the surface of the seed for introducing their stylets. Sections of the seeds *in situ* with the stylets of the feeding insects show that, in many cases, the stylets extend into superficial layers of the seedcoat i.e. into the outer integument, and they easily get detached from the seed as soon as the beak of the feeding insect is clipped off. In a number of other cases stylets extend deeper into the seedcoat, reaching the parenchymatous layer of the inner integument. These stylets remain intact with the seed after they are clipped off. Sometimes, however, the stylets may be introduced into or in-between the bases of the fuzzy hairs present on the surface of the delinted seed. *Oxycarenus* has never been found introducing its stylets into the seed beyond the seedcoat into the kernel or embryo.

#### CHIEF NUTRIENTS AVAILABLE IN HOST TISSUES

The nutrients required for the growth and maintenance of insects, like those for other animals, can be grouped under the following categories: Proteins and amino acids, carbohydrates, fats, minerals, and accessory growth factors like vitamins, sterols etc. Requirements for these substances vary with different species of insects. Minerals and accessory growth factors are mostly in such a form that they can be readily absorbed and utilised by the insects. The nutrients of the first three categories, however, may or may not be in a diffusible form. In case they are nondiffusible they must be brought into diffusible form by the action of digestive enzymes before they can be absorbed and utilised. In case an insect does not possess suitable enzymes it cannot utilise corresponding substrates. Since main object of the present study is to examine the correlation between the nutrients available to *Oxycarenus* in its preferred host-tissues and its ability to utilise those nutrients, it is necessary to have information on various nutrients occurring in the preferred host-tissues. Only the nutrients belonging to the first three of the above mentioned categories will be considered because they may or may not need enzymatic action prior to absorption. Minerals and vitamins etc. can be readily absorbed and are, therefore, not being taken into consideration here.

Chemical composition of the different parts of the cotton plant has been determined by a number of workers and their results have been included in reviews by Brown (1938), Leahy (1948), Tharpe (1948), Boatner (1948), Dollear and Markley (1948) and Dunning (1948). Table II, partially taken from Brown (1938), summarises the data on chemical composition of different parts of the mature cotton plant. It is apparent from the table that relative concentration of various nutrients in different parts of the plant is in the following order:

Carbohydrates: leaf > stem, root > seed boll > lint.

Proteins: seed > leaf > boll > stem, root > lint.

Fats: seed > boll > leaf > root > stem > lint.

Such data, however, do not give any information whether the proteins reported upon are all in the form of *native* proteins or free amino acids or both. Similarly it does not give any idea as to what poly-, oligo-, or monosaccharides are included in the total carbohydrates determined. In order to gain all this information free amino acids and sugars in the cottonseed hull and leaf were determined and the results will be presented in due course.

It may be further noted that *Oxycarenus* prefers to feed on one or the other tissue of cottonseed or of leaf. Therefore, chemical composition of entire seed or leaf, given in Table II, will not give much information on the nutrients available to the insect. For this purpose a knowledge of the constituents of different tissues of cottonseed and leaf is important.

So far as the leaf is concerned, it may be recalled that insects draw food-sap from almost all the tissues of the leaf except from the oil-glands. Therefore, constituents like gossypol, ethereal oils, tannins, resins, pigments etc., present in these glands (Brown, 1938), will not be available to the insects. Xylem vessels, on which the insects rarely feed, are known to contain mostly water and inorganic solutes. Most of the proteins, amino acids, starch, sugars and fats, present in the leaf, are distributed amongst the rest of the tissues, particularly phloem and mesophyll tissues, on which the insects mainly feed.

TABLE II

*Chemical Composition of Mature Cotton Plant (Brown, 1938)*

| Part of the Plant | Ash   | Protein | Fat   | Carbohydrates |
|-------------------|-------|---------|-------|---------------|
| Roots             | 3.72  | 3.00    | 2.78  | 49.88         |
| Stem              | 3.09  | 4.00    | 1.11  | 46.49         |
| Leaves            | 12.55 | 14.06   | 8.49  | 56.19         |
| Seed              | 3.65  | 22.13   | 23.05 | 39.26         |
| Lint              | 1.25  | 1.12    | 0.61  | 10.00         |
| Bolls             | 4.74  | 11.44   | 9.81  | 29.07         |

As regards the identity of sugars present in the leaf, Mason and Maskell (1928) reported that concentration of sucrose and reducing sugars of the leaf-sap undergoes diurnal variation—their quantities increasing during the day and decreasing during the night. Since *Oxycaenus* feeds on cotton leaf during the day as well as during night fluctuations in sugar content of the leaf will not affect their ingestion by the insect. However, Mason and Maskell (1928) did not examine whether sugars of cotton leaf included oligosaccharides other than sucrose; also, they did not establish the identity of reducing sugars present in the leaf. Results of the present study indicate that cotton leaf contains only three different sugars, namely, sucrose > glucose > fructose. Besides sugars, the following free amino acids are also present in cotton leaf: aspartic acid, histidine, serine, threonine and tyrosine.

Studies on the chemical composition of different parts of cottonseed by various workers have revealed that the chemical constituents undergo a great change in their distribution and concentration in different tissues of the seed during its development. Most of these changes are complete by the time the cotton boll opens. After its opening there may be a slight change in the concentration of certain constituents, particularly in the kernel, but no change in the distribution of various constituents amongst different tissues (Tharpe, 1948). Since, in the normal course, the cottonseed becomes available to *Oxycaenus* only when it is ripe and the boll has opened, a knowledge of the nutrients available to the insect can be gained only from the chemical composition of mature cottonseed.

Although kernel of the cottonseed is vary rich in proteins, oils and sugars like raffinose, the seedcoat or hull, from which the insect draws the food-sap, is quite poor in these constituents. Information on the chemical constituents of the hull has been incorporated in reviews by Dunning (1948), Leahy (1948), Tharpe (1948), Dollear and Markley (1948). Dunning (1948) has compiled data on the composition of cottonseed hull from several sources, which is given in Table III.

TABLE III

*Chemical Composition of Cottonseed Hull (Dunning, 1948)*

| Constituent               | Average % weight. Oven-dry basis |
|---------------------------|----------------------------------|
| $\alpha$ -Cellulose       | 43.9                             |
| Cross and Bevan Cellulose | 46.9                             |
| Pentosans                 | 29.5                             |
| Lignin                    | 21.95                            |
| Ash                       | 1.79                             |
| Protein                   | 3.30                             |
| Crude fibre               | 49.19                            |

Pentosans and sugars have been reported to occur in the parenchymatous cells of the hull (Leahy, 1948). Galactose and traces of sucrose are the only sugars reported to occur in the seedcoat (Dollear and Markley, (1948). The fat content of the hull, like that of proteins (3.3 per cent), is very low (0.9 per cent) (Guthrie *et al.*, 1944). In addition to these, the hull also contains certain free amino acids and sugars presence of which was qualitatively determined in the present work. The free amino acids present are alanine, asparagine, aspartic acid, glycine, methionine, serine, tryptophane and two unidentified amino acids. The sugars present in the seed hull are : raffinose > glucose > sucrose > fructose. No galactose was found in the hull.

#### DIGESTIVE ENZYMES

Digestive organs of *Orycaenus hyalinipennis*, like those of *Dysdercus koenigii* (Saxena, 1955) and some other Heteropterous insects, include a pair each of principal and accessory salivary glands, foregut, midgut and hindgut. The midgut is the longest division of the alimentary canal and is differentiated into three distinct regions: a wide, sac-like, *first ventriculus*, a narrow, tubular, *second ventriculus*, and a short, bulbous, *third ventriculus*. The hindgut is very short and not further divisible into intestine and rectum. Gastric caeca are not present in this insect.

Distribution of various enzymes in different parts of the digestive tract is given in Table IV. Of the enzymes tested for, cellulase, hemicellulases, inulase,  $\beta$ -glucosidase,  $\beta$ -galactosidase, polypeptidase and lipase were found to be completely absent. The remaining enzymes were detected in one or the other region of the digestive tract and are described below.

*Amylase.* Strong amylolytic activity was always detected in the salivary glands, all the regions of the midgut, and the hindgut. Although maximum activity occurred at pH 7.2, appreciable activity was also evident at pH 5.4. At pH 3.0, however, no amylolytic activity was detected at all.

*Maltase.* This enzyme was detected in the salivary glands and in all the three regions of the midgut but not in the hindgut. Its activity could be detected only at pH 5.4. Even at this pH value the enzymatic activity was quite weak since only a little quantity of glucose was liberated after an incubation of 36 hours; most of the substrate remained undigested.

*$\alpha$ -galactosidase.* Both raffinose and melibiose were separately used as substrates to detect the presence of this enzyme. Paper chromatograms revealed the presence of galactose in the incubated mixtures containing extracts of salivary glands and of the three regions of the midgut. This clearly indicates the presence

of an  $\alpha$ -galactosidase in each of the region mentioned above. Activity of the enzyme was detected at pH 5.4 but not at pH 3.0 or 7.2. It may be noted that in the incubated mixtures containing raffinose as the substrate no fructose or glucose was liberated as a result of hydrolysis of the trisaccharide. Instead, sucrose was detected in addition to galactose.

*Invertase.* In order to detect and characterise the enzyme invertase in the digestive tract of *Oxycarenus*, sucrose, melezitose and raffinose were separately used as substrates. With sucrose as the substrate, hydrolysis of the sugar, yielding glucose and fructose, was obtained with extracts of salivary glands and first and second ventriculi. This indicated presence of invertase in these regions. In the third ventriculus presence of this enzyme was detected only occasionally. In the hindgut also its occurrence was found to be quite irregular. In all experiments, maximum activity of the enzyme occurred at pH 5.4; very little activity occurred at pH 7.2 and none at pH 3.0. In comparison to other carbohydrases detected in the digestive tract of *Oxycarenus* the concentration of invertase was noticed to be much greater.

TABLE IV

*Distribution of enzymes in salivary glands and alimentary canal of Oxycarenus hyalinipennis (Costa)*

| Enzymes                   | Salivary glands | 1st Ventri- culus | 2nd Ventri- culus | 3rd Ventri- culus | Hindgut |
|---------------------------|-----------------|-------------------|-------------------|-------------------|---------|
| Amylase                   | +++             | +++               | +++               | +++               | +       |
| Maltase                   | +               | ++                | ++                | ++                | —       |
| $\alpha$ -galactosidase : |                 |                   |                   |                   |         |
| Acting on raffinose       | +               | +++               | ++                | ++                | —       |
| Acting on melibiose       | ++              | +++               | +                 | —                 | —       |
| Invertase :               |                 |                   |                   |                   |         |
| Acting on Sucrose         | ++              | +++               | ++                | —                 | ±       |
| Acting on melezitose      | +               | +++               | +                 | +                 | —       |
| Trehalase                 | —               | +++               | ++                | —                 | —       |
| Proteinase                | ++              | +                 | +                 | —                 | —       |
| Esterase                  | ++              | ++                | ++                | ++                | +       |

*Note.*—(i) *Plus* sign indicates the presence and *minus* the absence of the enzyme;  $\pm$  indicates presence of traces of the enzyme.

(ii) The number of plus signs gives an approximate estimate of the concentration of the enzyme in different parts of the gut.

(iii) The enzymes cellulase, hemicellulase, inulase,  $\beta$ -galactosidase,  $\beta$ -glucosidase, polypeptidase, and lipase were totally absent.

When the gut extracts were incubated with melezitose, instead of sucrose, hydrolysis of the substrate occurred in the extracts of the same regions which showed hydrolysis of sucrose. The hydrolysis of melezitose also occurred at pH 5.4 only, yielding glucose and turanose. This again must be due to the activity of invertase. With raffinose as the substrate only galactose and sucrose were found to be the hydrolytic products, as reported above. No fructose was liberated, which shows that the enzyme concerned with the digestion of raffinose is an  $\alpha$ -galactosidase only.



*Trehalase.* Activity of this enzyme was detected only in the first and second ventriculi at pH 5.4. At pH 7.2 slight activity of the enzyme was noticeable in the first ventriculus but not in the second. No activity was detected at pH 3.0.

*Proteinase.* Fairly strong proteolytic activity was noted in the extracts of salivary glands, first and second ventriculi but none in the third ventriculus and hindgut. In the extract of salivary glands the activity of the enzyme was greater at pH 7.2 than at pH 5.4. Even then fairly good activity was noticeable at pH 5.4. In the extracts of the first two ventriculi no difference in the activity of the enzyme was perceptible between pH 5.4 and 7.2. However, at pH 3.0 only slight proteolytic activity was detected.

*Esterase.* Enzyme capable of splitting up lower esters of fatty acids was detected in all the regions of the digestive tract.

### DISCUSSION

As mentioned before, there has been some diversity of opinion regarding the feeding behaviour of *Oxycarenus* on the cotton plant. According to some workers (Sickenberger, 1890; Willcocks, 1906 etc.) the insect feeds on seeds as well as on buds, flowers and young bolls of cotton. Peacock (1913) reports that *Oxycarenus*, when infesting cotton plant, feeds almost exclusively on seeds; but, on some other host plants, such as *Hibiscus* species, it feeds on almost all parts of the plant. Kirkpatrick (1923) has given an exhaustive discussion on the site of feeding of *Oxycarenus*. According to him all the stages of this insect "feed solely on the cottonseed", piercing "the testa of ripe seeds with their setae and extracting the juices of embryo". He also reports that adults and nymphs "may be seen sucking at the gland on the under surface of the midrib of the leaf, near the base; but in the writer's (Kirkpatrick's) opinion this is merely a means of obtaining moisture in the absence of dew". He also reports that the bugs may draw moisture from the glands of epicalyces as well. He does not clarify whether the glands he refers to are the 'oil-glands' or the nectaries present on the undersurface of large veins of the leaf.

It may be remarked that Kirkpatrick (1923) makes no mention of the method he adopted to determine the relative preference of *Oxycarenus* for different parts of cotton plant and for the determination of tissues of the seed or leaf from which, he claims, the insect draws its food-sap. Furthermore, he does not give any data nor any experimental evidence in support of his conclusions regarding the site of feeding of the pest. The only evidence he cites in support of his views is the loss in weight of cottonseeds from plants infested with *Oxycarenus*. This sort of evidence is very much indirect and cannot be relied upon since a number of other factors may be responsible for the loss in weight of cottonseeds in the fields.

Experimental investigations of Saxena and Krishna (1958) have indicated that *Oxycarenus* prefers to feed on green parts, particularly the leaf, of cotton plant much more than on mature seed. Results of the present study go a step farther to contradict the conclusion of Kirkpatrick (1923). Sections of cotton leaf *in situ* with the stylets of insects feeding on it clearly show that the stylets penetrate deeper than the epidermis, extending mostly up to mesophyll or phloem tissues and rarely to xylem vessels. They have never been observed to extend into the nectaries or into the oil-glands scattered over the leaf. This observation is in conflict with the view of Kirkpatrick (1923) that the insect goes to leaf only for getting moisture from the glands. Similarly, sections of cottonseeds *in situ* with the stylets of insects feeding on it show that the insect never penetrates its stylets into the seed deeper than the seedcoat or the hull and, therefore, its stylets can never reach kernel or embryo of the seed as contended by Kirkpatrick (1923). It appears that very hard texture of the seedcoat also plays an important rôle

in inhibiting its complete penetration by the stylets which can extend up to the parenchymatous tissues of the inner integument of the seedcoat.

The facts presented in this paper make it possible to consider the correlation between the nutrients available in cottonseed or leaf and the ability of *Oxycaenus* to utilise those nutrients. It has been observed that the insects feeding on cotton leaf draw food-sap from both mesophyll and phloem tissue at one time or the other. They rarely suck juice from xylem vessels and never from the oil-glands. In view of this, substances like gossypol, etheral oils, resins, tannins, flavones, etc. (Brown, 1938), which occur in oil-glands and which are specific to cotton and certain other Malvaceous plants, will not be available to the insects. According to our present knowledge of plant physiology xylem vessels contain mainly water and minerals which, therefore, will be available to the insects only occasionally. Most of the proteins, free amino acids, starch, sugars and fats of the leaf are distributed amongst the mesophyll and phloem tissues and will, therefore, be mostly available to the insects feeding on cotton leaf.

As to the nutrients available to insects feeding on cottonseed, it may be noted that the seed is rich in proteins, fats and sugars (Table II) but these are mostly concentrated in the kernel. The seedcoat, from which the insects draw food-sap, is very poor so far as proteins (3.3 per cent; Dunning, 1948) and fats (0.9 per cent; Guthrie *et al.*, 1944) are concerned. The carbohydrates, in addition to cellulose, present in the seedcoat are: pentosans (29.5 per cent; Dunning, 1948), raffinose, sucrose, glucose and fructose (vide p. 252). Small amounts of free amino acids also occur in the seedcoat (p. 252).

Of the constituents of cotton leaf and seedcoat, reported above, free amino acids and sugars like glucose and fructose are in a diffusible form and can be readily utilised by the insect. Substances like proteins, starch, pentosans, sucrose, raffinose and fats are non-diffusible and have to be digested before they can be absorbed. Digestive enzymes present in the digestive tract of *Oxycaenus* are: proteinase, amylase, maltase, invertase,  $\alpha$ -galactosidase and an esterase. Their presence bestows upon the insect ability to digest proteins starch and sucrose available in the leaf and also, to digest raffinose, sucrose and slight amount of proteins available in the seedcoat. Fats present in the leaf and seedcoat cannot be utilised by *Oxycaenus* due to the absence of lipase. Similarly, pentosans present in the seedcoat cannot be utilised owing to the lack of any hemicellulase. Absence of cellulase in the insect rules out the possibility of any digestion of cellulose.

It may be noted that cotton leaf contains a much greater proportion of proteins, carbohydrates and fats (Table II) than the seedcoat (Table III). Free amino acids and sugars are the only constituents of the seedcoat which may be utilised by *Oxycaenus* for its nourishment. However, it is noteworthy that the insects feeding on seedcoat have access to raffinose which is not available in the leaf. It is difficult to say whether ingestion of raffinose is in any way important in the nutrition of *Oxycaenus*.

These facts appear to show that, so far as proteins, carbohydrates, and fats are concerned, leaf is much better a source of food for *Oxycaenus* than cottonseed hull.

On the basis of some of the results presented in this paper, nature of invertase present in the gut of *Oxycaenus* may be considered. Two types of invertases are known to occur in different organisms:  $\beta$ -*h*-fructofuranosidase and glucosaccharase (Kühn, 1923 *a, b*). Both of these enzymes can act on sucrose yielding glucose and fructose as the initial products; but the mechanisms of their action is different. The first enzyme acts on sucrose by attacking fructose moiety of the molecule. The same enzyme is capable of acting on raffinose (6- $\alpha$ -*d*-galactopyranosyl- $\alpha$ -*d*-glucopyranosyl- $\beta$ -*d*-fructofuranoside), liberating fructose and melibiose, since in this trisaccharide fructose part of the molecule is again free and accessible to the enzyme. However, the enzyme cannot act on melezitose

(3- $\alpha$ -*d*-glucopyranosyl- $\beta$ -*d*-fructopyranosyl- $\alpha$ -*d*-glucopyranoside) in which the fructose moiety of the molecule is blocked by glucose.

The second type of enzyme i.e. glucosaccharase, also termed  $\alpha$ -*n*-glucosido-invertase (Neuberg and Mandl, 1950) is an  $\alpha$ -glucosidase (Gottschalk, 1950) which attacks sucrose from the free glucose end of the molecule. It can also act on melezitose where the glucose part of the molecule is free. On the other hand, raffinose, where glucose moiety is blocked by galactose, cannot be acted upon by glucosaccharase. The fact that invertase present in *Orycaenus* acts upon sucrose and melezitose and not on raffinose indicates that the enzyme concerned is glucosaccharase and not  $\beta$ -*h*-fructofuranosidase.

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# EFFECT OF HORMONE HERBICIDES ON PADDY (*ORYZA SATIVA* L.)

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## ABSTRACT

The effects of four hormonal herbicides viz. amine, sodium salt and ester of 2, 4-D and M.C.P.B. in 1,000, 2,500 and 5,000 p.p.m. concentration on 'Aman' variety of paddy have been investigated. The relation between the age of the crop and the susceptibility to hormone herbicides has also been studied by giving the treatments at 'early', 'pre-flowering' and 'post-flowering' stages.

It has been found that all the chemicals produce a certain degree of adverse effect on the growth of paddy, seed-sterility and yield. Unlike wheat, paddy has been found to be more susceptible to M.C.P.B. than 2,4-D.

It has further been found that irrespective of the chemical and the stage of treatment, reduction in the rate of growth and yield and increase in the frequency of ear abnormalities is proportional to the concentration of herbicide.

It has been observed that the growth is most affected when the treatment is made at the early stage, while the yield is minimum at the pre-flowering stage of treatment. The low yield and high percentage of seed sterility has been found to coincide, and hence it is assumed that perhaps the herbicide interferes with the seed-setting in paddy.

Besides, a large number of morphological abnormalities of the ear have been recorded. Since these abnormalities occur almost exclusively when treated at the pre-flowering stage, they have been attributed largely to the formative disturbances caused by the hormone herbicides.

From the present investigations, it has been concluded that the most suitable stage for the application of the hormone herbicides for controlling weeds in paddy is the early stage of the crop growth and treatment at pre-flowering stage must always be avoided. However, in crops where there is a considerable difference in the time of flowering of weed and the crop, it has been suggested that herbicide treatment at the pre-flowering stage of the weeds is likely to exert an effective control for the next season by reducing the setting of seeds.

## INTRODUCTION

It is the property of selective phytotoxicity of hormone herbicides, which makes possible the chemical control of weeds in agriculture. But, whereas the application of such chemicals either retards the growth or completely eradicates most of the broad-leaved plants, it does not leave the narrow leaved ones entirely unaffected. In monocot crops although these effects might not be very detrimental from the viewpoint of the yield, a complete knowledge of the various factors involved in chemical weed control is essential. Besides, it has been recorded that the susceptibility of the plant species varies with their age. In other words, the physiology of the plants seem to bear a direct relationship with the effect of the herbicides. Unfortunately, no work has so far been done in this line in the Indian crop plants.

It, therefore, seemed desirable to study the effect of some of the common hormone herbicides on paddy at various stages of growth of the crop, in an attempt to find out the relative toxicity of the different chemicals, the most suitable stage of growth for the application of the weedicides, as well as to study the different deleterious effects produced, their frequency and significance in relation to the yield of paddy.

## MATERIAL AND METHOD

The experiment was conducted on the field scale, at the Field Experimental Station of Bose Research Institute, at Shyamnagar (24 Parganas), on the transplanted "Aman" paddy in a suitably planned lay-out. The treatments were made at three different stages of growth. viz.,

- (i) Early (about 6 weeks after germination)
- (ii) Pre-flowering (about 11 weeks after germination)
- (iii) Post-flowering or milk seed (about 12-13 weeks after germination).

Four different herbicidal products, namely, the amine salt of 2, 4-dichlorophenoxy acetic acid (amine 2, 4-D), the butyl ester of 2, 4-D (Ester 2, 4-D), the sodium salt of 2, 4-D (Na-2, 4-D) and methyl, chloro-phenoxy butyric acid (M.C.-P.B.) were used in different concentrations of 1,000, 2,500 and 5,000 p.p.m. The chemicals were sprayed in form of aqueous solutions or emulsions, using a constant pressure, high volume, Knapsack type of spraying equipment. A uniform rate of spraying at about 80 gallons of solution per acre was maintained in all the treatments. Thus on an average, the treatment given was slightly higher than the usual so as to get pronounced effects of herbicidal injury, if any.

The data with regard to growth, flowering, and sterility, etc. were taken from 10 plants selected at random from each subplot. The data of the yield were taken from each subplot separately and compared with the 'control'.

## EXPERIMENTAL RESULTS

*Initial effects of the treatments :*

The initial effects of the treatment on paddy included the wilting, yellowing and drooping of the plants. Paddy is essentially resistant to a normal dose of herbicidal chemicals at which most of the dicot weeds succumb. In the present set of treatments the initial injury varied with the concentration as well as the age of the crop. However, in none of the concentrations, the initial effect of the treatment lasted for more than 2-5 days and the plants recovered thereafter. The maximum injury was recorded in case of 5,000 p.p.m. treatments. Again the initial effect of the herbicides was most pronounced when the application was made at the early stage of the growth of plants. With the increasing age of the plants, there was lesser injury and the recovery was relatively quicker.

*Effect on the growth :*

Although the initial effects of herbicides disappear soon, more lasting effects are produced on the physiology of plants which express in the less height of the plant and other morphological abnormalities.

The growth of the plants in each treatment was taken in terms of the maximum height attained by the plants at the mature stage. The height of ten plants selected at random from each subplot was taken and the data are included in Table I.

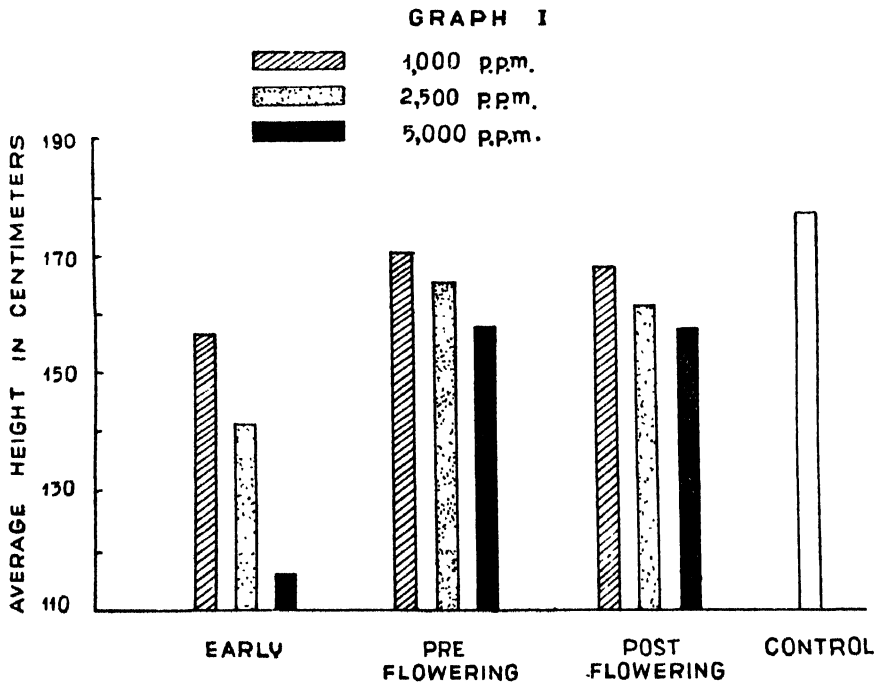
From the table below, it will be seen that in all the treatments there was a retardation of the growth i.e. the average height of the plants in all the treatments was lower than the control. On analysing the data with the help of Graph I, it was seen that like the initial effects, the effect of the herbicides on the growth was most pronounced at the early stage, whereas there was no significant difference between the various treatments at the pre-flowering and post-flowering stages. Even the differences between the effects of the higher concentrations were not much pronounced at the latter stages. In other words, the height of the paddy treated at a relatively mature stage with 1,000 p.p.m. almost corresponds with

that of the control and the height at higher concentrations, viz., 2,500 and 5,000 p.p.m. is not very much lower.

TABLE I  
*Max. height of plants in cm. (Average of 10 plants)*

| Treatment \ Stage | Early        |              |              | Pro-flowering |              |              | Post-flowering |              |              | Mean  |
|-------------------|--------------|--------------|--------------|---------------|--------------|--------------|----------------|--------------|--------------|-------|
|                   | 1,000 p.p.m. | 2,500 p.p.m. | 5,000 p.p.m. | 1,000 p.p.m.  | 2,500 p.p.m. | 5,000 p.p.m. | 1,000 p.p.m.   | 2,500 p.p.m. | 5,000 p.p.m. |       |
| Amine 2, 4-D      | 155.6        | 140.0        | 140.0        | 178.0         | 176.0        | 164.0        | 174.0          | 171.0        | 165.0        | 162.4 |
| Ester 2, 4-D      | 164.0        | 147.0        | 122.4        | 178.0         | 177.0        | 170.0        | 174.0          | 172.0        | 170.0        | 164.6 |
| Na-2, 4-D         | 153.0        | 127.6        | 86.8         | 161.5         | 156.0        | 140.0        | 160.0          | 151.0        | 140.0        | 141.7 |
| M.C.P.B.          | 155.8        | 137.4        | 114.0        | 168.0         | 162.0        | 154.0        | 165.5          | 160.0        | 154.0        | 152.3 |
| Control           | —            | —            | —            | —             | —            | —            | —              | —            | —            | 175.0 |
| Mean              | 157.1        | 137.75       | 115.8        | 171.4         | 167.75       | 157.0        | 168.37         | 163.5        | 157.25       | —     |

There is, on the other hand, a pronounced depression of growth when the treatment was made at the early stage. Here the height is considerably lower than the control even at 1,000 p.p.m. and with the increasing concentrations, there is a steep reduction in the height of the plants; being on the average only 115.0 cm. at the 5,000 p.p.m.



This shows that there exists some sort of relationship between growth and the age of the paddy as well as the strength of the chemical. The nature and the consequence of this relationship will be discussed later.

*Effect on the flowering :*

The date of the first flowering in the same ten plants selected at random from each subplot was taken and the duration for flowering was calculated from it. It has been found that on an average, the treatment with herbicides produced no remarkable effect on the time of flowering.

*Effect on the Ears :*

(a) *The length of the ear :* The length of the ears was taken from each treatment in an attempt to determine whether the depression of the vegetative activity has any corresponding influence on the reproductive activity of the plant and is included in Table II.

TABLE II

*Length of the ears (av. of 10 ears in cm.)*

| Treatment \ Stage | Early        |              |              | Pre-flowering |              |              | Post-flowering |              |              | Mean  |
|-------------------|--------------|--------------|--------------|---------------|--------------|--------------|----------------|--------------|--------------|-------|
|                   | 1,000 p.p.m. | 2,500 p.p.m. | 5,000 p.p.m. | 1,000 p.p.m.  | 2,500 p.p.m. | 5,000 p.p.m. | 1,000 p.p.m.   | 2,500 p.p.m. | 5,000 p.p.m. |       |
| Amine 2, 4-D      | 23.8         | 20.8         | 19.6         | 26.4          | 24.4         | 24.4         | 25.0           | 23.4         | 23.5         | 23.4  |
| Ester 2, 4-D      | 22.75        | 22.4         | 17.7         | 25.2          | 23.9         | 22.9         | 26.9           | 26.0         | 25.05        | 23.6  |
| Na-2, 4-D         | 24.3         | 23.1         | 20.05        | 25.5          | 24.15        | 25.1         | 26.6           | 25.8         | 23.4         | 24.2  |
| M.C.P.B.          | 25.1         | 23.35        | 20.9         | 25.5          | 25.7         | 25.5         | 25.8           | 24.6         | 26.4         | 25.87 |
| Control           | —            | —            | —            | —             | —            | —            | —              | —            | —            | 26.7  |
| Mean              | 23.99        | 22.4         | 19.56        | 25.65         | 24.5         | 24.5         | 26.07          | 24.9         | 24.59        | —     |

From the table it will be seen that as in the case of growth, the length of the ears was affected to a certain degree as a result of treatment with hormone herbicides. Although the responses due to different chemicals do not show significant differences, there is a clear relationship between the age of the plants at the time of treatment and the concentration of the treatment. It was noticed that the effect of the treatments was to reduce the length of the ears to a certain extent when treated at the early stage of growth which was proportional to the concentration of the chemical used. Thus the 5,000 p.p.m. treatment has the lowest average length of the ear i.e., 19.56 cm. as against 26.7 cm. of the 'control'.

The effects of the reduction of the size of the ears will be discussed later.

(b) *Induced morphological abnormalities of the ear :* Besides the reduction in the length of the ears, a large variety of malformations of the ear were recorded in the various treated plots in course of the observations. These included—

(1) Failure of the inflorescence to emerge from the sheath. Figs. 1, 2 and 3 represent the various stages of the arrest of inflorescence. Such suppression of the ears following the herbicide treatment has been recorded by Unrau and Larter (1952) in wheat and Scragg (1952) in barley, oats and wheat. Scragg has termed



some of these as 'Bowed Ears', (2) Epinasty of the inflorescence axis : Epinasty of the inflorescence axis results in the formation of 'Screwed' and sometimes 'hooked' ears. Such ears have also been recorded in the present observation (Fig. 4). It will be noted that in such cases, the development of the ear is more or less unilateral. (3) 'Feathery Ears' : In two rare cases, the inflorescence was found to be erect and open presenting a feathery appearance, similar to certain other members of Graminae like *Saccharum* sp., *Imperata* sp., etc. Fig. 5 represents a normal ear of paddy while Fig. 6 shows the spread out 'Feathery' ear.

#### *Effects on the ripening of the crop :*

Like the flowering, the time of ripening of paddy was recorded for each subplot. It has been found that on the average, the ripening of the crop remained more or less unaffected in spite of the treatments.

#### *Effect on the Seed sterility :*

An interesting observation, in course of the present investigation was the effect of the herbicides on the seed setting. It was found that in certain treatments, a large number of seeds from each ear remained empty. Therefore, ten plants selected at random (referred to earlier) from each subplot were harvested separately. The data regarding the total number of sterile seeds in each case were collected. On the basis of this, the percentage of seed sterility has been determined and is included in Table III.

TABLE III  
Percentage of seed sterility

| Treatment    | Stage | Early        |              |              | Pre-flowering |              |              | Post-flowering |              |              | Mean  |
|--------------|-------|--------------|--------------|--------------|---------------|--------------|--------------|----------------|--------------|--------------|-------|
|              |       | 1,000 p.p.m. | 2,500 p.p.m. | 5,000 p.p.m. | 1,000 p.p.m.  | 2,500 p.p.m. | 5,000 p.p.m. | 1,000 p.p.m.   | 2,500 p.p.m. | 5,000 p.p.m. |       |
| Amine 2, 4-D |       | 11.32        | 13.49        | 16.20        | 49.70         | 14.30        | 92.56        | 52.68          | 42.26        | 46.15        | 40.96 |
| Ester 2, 4-D |       | 15.64        | 25.15        | 19.30        | 48.33         | 26.02        | 75.29        | 41.40          | 43.10        | 45.52        | 37.64 |
| Na-2, 4-D    |       | 9.89         | 10.37        | 8.28         | 78.35         | 87.22        | 88.23        | 14.82          | 34.79        | 53.18        | 42.19 |
| M.C.P.B.     |       | 19.8         | 14.18        | 20.99        | 19.01         | 61.75        | 72.58        | 19.96          | 43.25        | 50.94        | 35.83 |
| Control      |       | —            | —            | —            | —             | —            | —            | —              | —            | —            | 14.1  |
| Mean         |       | 14.16        | 15.79        | 16.19        | 48.85         | 54.82        | 82.16        | 32.21          | 40.83        | 48.95        | —     |

From the table, it will be seen that there is a marked difference in the sterility of seeds, not only at the various stages of growth but also due to different chemicals used in this experiment. In other words, the highest percentage of sterility is recorded in the application made at the pre-flowering stage (ref. Graph II), followed by the post-flowering stage. The minimum effect is produced when the application is made at the early stage.

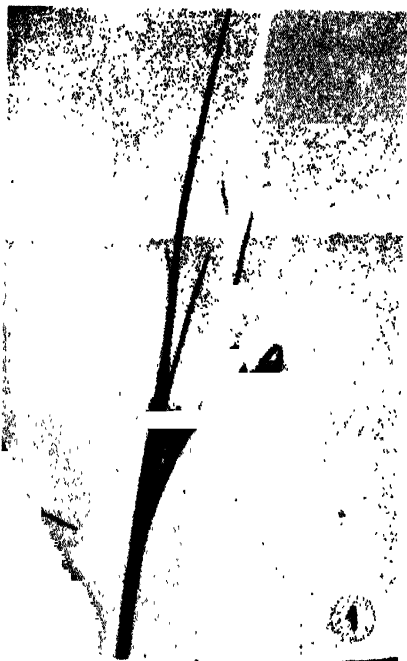


Fig. 1.—*Photograph showing an arrested ear of paddy in a plant treated with 2,500 p.p.m. of amine 2, 4-D at the pre-flowering stage.*

Fig. 2.—*Photograph showing a partly exposed ear in 5,000 p.p.m. of amine 2, 4-D.*

Fig. 3.—*Photograph showing a short ear showing the effect of the stiffness of the 'sheath' on its development*

Fig. 4.—*Photograph showing a 'bowed' ear showing the epinasty of the inflorescence axis and few seeds*



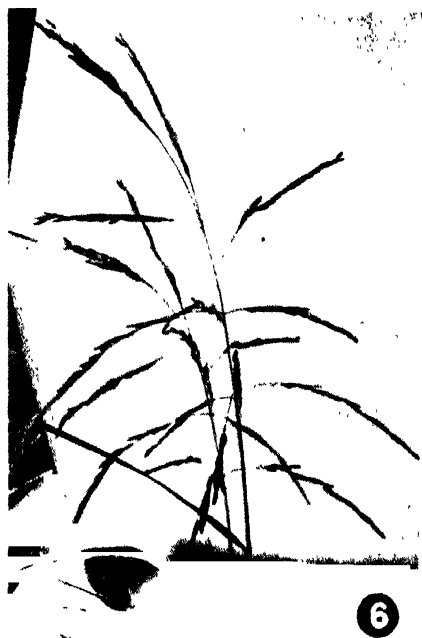
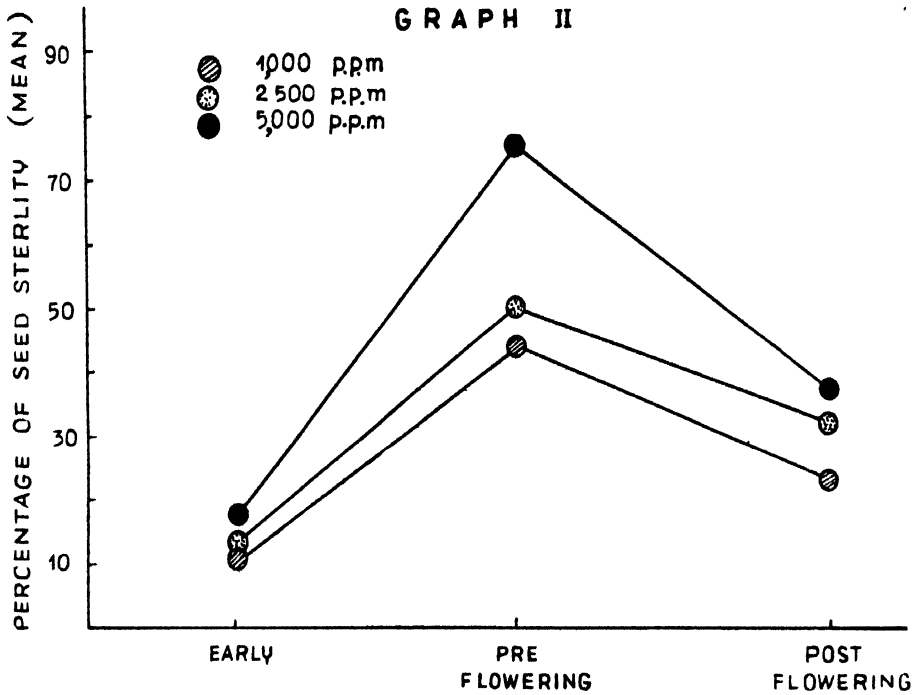


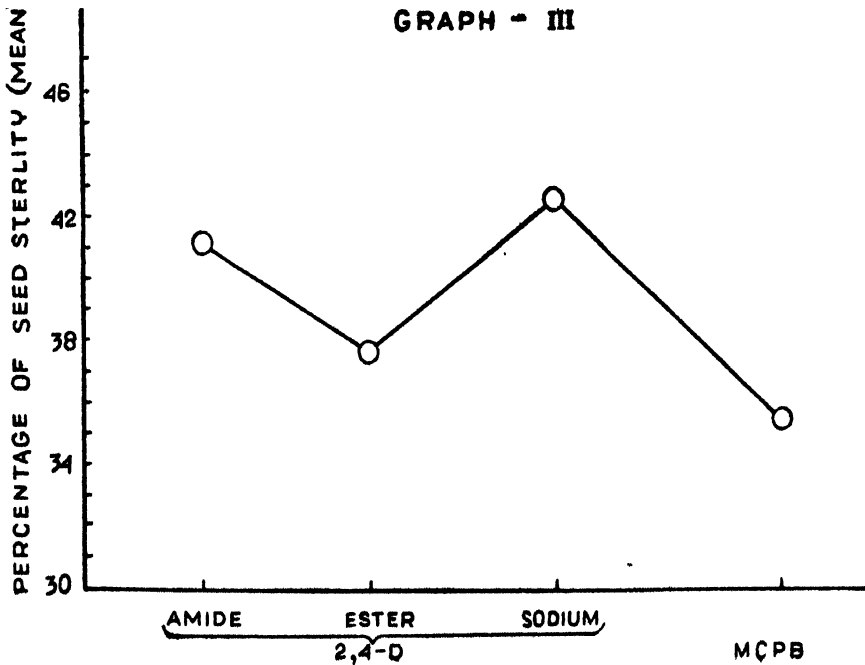
Fig. 5.—*Photograph showing a normal ear of paddy (Control).*

Fig. 6.—*Photograph showing a "feathery" inflorescence from a plant treated with 2,500 p.p.m. of ester of 2, 4-D.*





When considering the differences between the effects of the different chemicals, it has been found that the effect of 2, 4-D group of chemicals is more than the M.C.P.B. (Graph III) and that amongst the 2, 4-D derivatives the highest sterility is caused by the sodium salt and lowest by the ester formulation.



Besides, the different concentrations of the herbicides also affect the percentage of seed sterility. The following two-way table denotes the relationship between the concentration of the treatment and the age of the plant irrespective of the chemical used.

TABLE IV  
*Percentage of seed sterility (conc : × stage)*

| Stage \ Conc : | 1,000 p.p.m. | 2,500 p.p.m. | 5,000 p.p.m. | Mean  |
|----------------|--------------|--------------|--------------|-------|
| Early          | 14.16        | 15.79        | 16.19        | 15.71 |
| Pre-flowering  | 48.85        | 54.82        | 82.16        | 61.94 |
| Post-flowering | 32.21        | 40.83        | 48.95        | 40.66 |
| Mean           | 31.74        | 37.14        | 49.1         | —     |

The above data suggest that on the average there is a direct relationship between the increase in the concentration of the treatment and the percentage of sterility induced. This can also be confirmed by Graph II. However, the effect is more pronounced in case of the treatments made at the pre-flowering stage—being 48.85 per cent, 54.82 per cent and 82.16 per cent for 1,000, 2,500 and 5,000 p.p.m. respectively. When compared to the 'control' all the chemicals seem to induce a high degree of seed sterility. The causes and the consequences of such sterility will be discussed later.

#### *Effect on the yield of Paddy :*

Last but economically by far the most important effect of the treatment with hormone chemicals is on the yield of paddy. As stated earlier, the yield of paddy in the various treatments was reckoned in terms of the yield per sub-plot.

When the data were statistically analysed, it has been found that 'control' gives significantly higher yield than all other treatments but there is practically no significant variation in the yields among the four different chemicals. The stage of growth at which the treatment is made has an effect on the yield of paddy as is observed from the marginal means of the Tables Nos. V and VII. Chemicals applied at early and post-flowering stages of the growth of the plant give higher yield than those treated at the pre-flowering stage. However, the differences between the yields of early- and post-flowering stages are not significant.

Next, the strength or the concentration of the chemicals seems to have an important part to play. From Table VI, it is observed that the yield decreases with the increasing concentration of the chemical. Thus at 1,000 p.p.m. almost all the chemicals give the highest yield as compared to the two other concentrations (2,500 and 5,000 p.p.m.).

All the possible interactions between the three factors i.e. chemicals, time of application and strength, were studied and have not been found to be significant.

TABLE V

*Mean yield in lb./plot*  
(Chemicals  $\times$  Time of application)

| Chemical \ Time |       |               |                |      |
|-----------------|-------|---------------|----------------|------|
|                 | Early | Pre-flowering | Post-flowering | Mean |
| Control         | —     | —             | —              | 5.50 |
| Amine 2, 4-D    | 2.25  | 1.29          | 2.43           | 1.99 |
| Ester 2, 4-D    | 2.19  | 0.94          | 2.92           | 2.02 |
| Na-2, 4-D       | 4.04  | 1.65          | 2.25           | 2.65 |
| M.C.P.B.        | 2.33  | 1.67          | 1.83           | 1.94 |
| Mean            | 2.70  | 1.38          | 2.36           | —    |

|                         |    |    |    |        |
|-------------------------|----|----|----|--------|
| S. E. of chemical       | .. | .. | .. | = 0.31 |
| S. E. of time           | .. | .. | .. | = 0.27 |
| S. E. body of the table | .. | .. | .. | = 0.54 |

TABLE VI

*Mean yield in lb./plot*  
(Chemical  $\times$  strength)

| Chemical \ Strength |              |              |              | Mean |
|---------------------|--------------|--------------|--------------|------|
|                     | 1,000 p.p.m. | 2,500 p.p.m. | 5,000 p.p.m. |      |
| Control             | —            | —            | —            | 5.50 |
| Amine 2, 4-D        | 2.54         | 2.60         | 0.83         | 1.99 |
| Ester 2, 4-D        | 2.58         | 1.98         | 1.49         | 2.02 |
| Na-2, 4-D           | 4.00         | 2.88         | 1.06         | 2.65 |
| M.C.P.B.            | 2.06         | 3.00         | 0.77         | 1.94 |
| Mean                | 2.80         | 2.01         | 1.04         | —    |

TABLE VII

*Mean yield in lb./plot*  
(Time  $\times$  strength)

| Strength \ Time |       |               |                |      |
|-----------------|-------|---------------|----------------|------|
|                 | Early | Pre-flowering | Post-flowering | Mean |
| 1,000 p.p.m.    | 3.67  | 1.84          | 2.88           | 2.8  |
| 2,500 p.p.m.    | 3.66  | 1.74          | 2.45           | 2.61 |
| 5,000 p.p.m.    | 0.79  | 0.58          | 1.75           | 1.04 |
| Mean            | 2.70  | 1.38          | 2.36           | —    |

|                            |    |    |    |        |
|----------------------------|----|----|----|--------|
| S. E. of time or strength  | .. | .. | .. | = 0.27 |
| S. E. of body of the table | .. | .. | .. | = 0.47 |



## DISCUSSION

*The relation between age and susceptibility of paddy :*

(a) *Growth* : Hormone herbicides generally produce a growth retarding effect on the plant unless used in very low concentrations. Sufficient work on this aspect as well as on the relative susceptibility of weeds has been done for the ultimate purpose of the control and eradication of such plants.

The growth inhibition recorded during the course of the present investigation has been incorporated in Table I and explained through Graph I. It has been observed that as regards growth, the inhibition of growth is one of the most outstanding features of the herbicide toxicity—a property which in its final form has given rise to the concept of chemical control of weeds.

An interesting observation during the course of present experiments is the presence of a definite relationship between the age or the stage of growth of the crop and the extent of the herbicide injury caused. As recorded earlier, irrespective of the herbicide used, the growth of paddy is markedly affected only in case of the treatments made at the early stage of growth. The maximum height attained in the other two sets of treatment viz., the pre-flowering and post-flowering stages does not vary appreciably from the 'control'. This suggests that at the early stage of growth, when the plant is in a very active state of growth, the application of hormone herbicides brings about certain irreversible physiological disbalance. This expresses itself in form of an inhibition or retardation of growth as compared with the 'control'. The disbalance itself may be caused either as a result of the destruction of the native auxins responsible for growth or due to an unhealthy competition for the active sites (on the substrates) as suggested by Foster *et al.* (1952).

In the other two stages, differentiation and reproduction rather than growth are the principal sites of enzymatic activity and hence the effect of the herbicides is not very pronounced in terms of the maximum height attained by the plants. In other words, it may be said that in the latter two sets of treatments, the plants having almost attained the maximum height, the effect of herbicide is not expressed to the same extent. The stunting of growth of the plant in turn affects the size of the ears. As will be seen from Table II, the ears are largest in the control and shortest in the plants sprayed at the early stage of growth. However, the differences in the length of the ears in the latter two treatments are not marked as compared with the 'control'.

(b) *Flowering and maturity* : The data regarding the time of flowering and maturity of crop at any stage of treatment do not vary much from the 'control'. This suggests that the treatment with hormone herbicides does not affect the photoperiodic response of paddy. The present observations are therefore not in conformity with those of Chakravarti and Pillai (1955) in *Brassica campestris* that the application of 2, 4-D and T.I.B.A. produce an earliness of flowering.

Similarly, Scragg (1952) has noted that the ripening of wheat, oats and barley is affected as a result of the treatment with selective herbicides. However, this observation also could not be confirmed by the present experiment, as the time taken for seed setting and ripening remained unaffected following the herbicide treatment.

(c) *Seed sterility* : Hormones are known to affect the fruit formation and seed-setting in plants. Thus De Tar *et al.* (1950) in pears and Osborne and Wain (1950) in apple, have reported increased fruit formation as a result of the application of 2, 4-5 trichlorophenoxypropionic acid. Increased fruit yield has also been reported in certain crops like tomato through the use of synthetic auxins. However, in most cases the increased fruit yield is not accompanied by a corresponding increase in the yield of seeds, which is explained on the basis of parthenocarpic development of the fruits. It has been suggested that the external

supply of auxin affects the fruit formation in two ways. Firstly, it meets the auxin deficiency in cases where the supply of native auxin acts as the limiting factor for the fruit formation. Secondly, the external supply of auxin sometimes provides the necessary stimulus for the growth and development of fruits, even though the usual pollination and fertilisation might not have taken place (parthenocarpy).

Despite the record of certain authors that even 2, 4-D group of chemicals not only increase the fruit yield but also enhance the seed-setting, the observations during the present investigations have been to the contrary. It has been noted that when application was made at the early stage of plant growth, there was no marked increase in the percentage of seed sterility over the control. The other two stages, i.e. pre-flowering and post-flowering, responded to the spray in form of increased seed-sterility, being maximum in the former. In the highest concentration, viz., 5,000 p.p.m., seed sterility at this stage has been recorded to be as high as 80 per cent.

The observations suggest that like the growth, the effect of the herbicides on the seed-setting is linked with the age of the plants. The lower effect at the early stage in this case may be explained on the basis of the absence of the inflorescence primordia at that stage and the sufficient period of recovery allowed between the treatment and the differentiation of the inflorescence.

The greatest damage as stated earlier, was caused when the application was made on about 13 week-old paddy—i.e. the pre-flowering stage. This can be explained on the basis of—

(a) The damage caused to the differentiating inflorescence resulting in the formation of fewer flowers and developmental abnormalities in flowers already formed.

(b) The sterility may also be caused due to the upsetting of the meiosis and pre- and post-meiotic mitoses both in the mega- as well as micro-sporogenesis.

Of the two possible effects noted earlier, it may be pointed out that although there is a marked deterioration in the number of fertile seeds, the total number of flowers formed per inflorescence is not conspicuously affected. Hence, the developmental abnormalities mentioned under (a) do not seem to have much bearing on the frequency of seed sterility.

Regarding the second cause, Srivastava (1958) has recorded a high frequency of meiotic abnormalities induced in *Crotalaria juncea* L., following the treatment of the inflorescence with hormonal herbicides. Although the meiotic abnormalities in the present material following the treatment have not been investigated, the presence of a large number of empty seeds suggests that the setting of the seeds rather than the formation of flowers has been responsible for the low yield.

In the third set of treatment, viz., at the post-flowering or milk-seed stages, there is high frequency of empty seeds as compared to the control and the plants treated at the early stage, but much lower than those treated at the pre-flowering stage. This suggests that the pollination having taken place, the further development of the seed is not affected due to the herbicidal treatment. But since all the flowers of any inflorescence are not mature at the same time, the unfertilised ovules as well as the immature flowers are affected by the treatment and contribute towards an increased percentage of seed sterility.

(d) *Yield*: The yield of paddy in 1b-plot has been described earlier and the data analysed on the basis of relation between the different factors are included in Tables V, VI and VII.

As stated earlier, it has been noted that the yield in all the treatments is lower than that of the 'control'. This suggests that when applied in herbicidal concentrations (as used in the usual weed control operations in paddy) the yield of paddy is adversely affected. However, a closer scrutiny of the data reveals that there exists a definite correlation between the age of the treatment and the consequent reduction in the yield. The yield varies between the three stages of application.

It is highest when treated at the early stage of growth, lower at the milk-seed stage and lowest at the pre-flowering stage. The variations in the yield between the pre-flowering stage and others are significant, although the variations between the early and milk seed stage are not very pronounced. This also corresponds with the frequency of the seed sterility in all the treatments. In other words, the present observations suggest that the reduction in yield at various stages of growth is probably a function of the seed sterility caused as a result of the treatment with hormone chemicals.

Rossman and Sprague (1949) in maize, Moore (1950) in wheat and Kent *et al.* (1957) in wheat and oats have similarly recorded that the yield is reduced following the treatment with various herbicidal products of the hormone group. The present data find further support in the works of Andersen and Hermansen (1950) and Scragg (1952) in wheat, oats and barley that the lowest yield is produced when the application is made at the time of differentiation of the inflorescence. The low yield also coincides with the highest frequency of morphological abnormalities of the ear, as very few have been recorded in treatments other than the pre-flowering. How far the statement of Andersen and Hermansen (1950) that the ear abnormalities do not produce any marked effect on the yield, is applicable to the present experiment could not be confirmed due to paucity of material.

Moore's suggestion (1950) that the reduction in yield was due to the tillering behaviour of the treated population could not be confirmed. His second suggestion that application at the flowering stage reduced yield due to reduction in grains per ear rather than ear/plant may now be further extended to be due to failure of seed-setting.

Again the retardation of the vegetative activity due to the application at the early stage of growth has a corresponding effect on the yield, but least as compared to the other two stages.

Considering the relationship between the age and susceptibility of paddy in general, it appears that although paddy is susceptible to the treatment with the hormone herbicides at almost all the stages of growth, the maximum deleterious effect on the yield is produced when the application is made at the pre-flowering stage. The applications made at the early and post-flowering stages do not affect the yield as much. Since the purpose of weed control, i.e., reducing the competition between the weeds and the crop plants in order to provide better conditions of growth, is hardly served if the weeds are killed after the crop has flowered, application at this stage does not seem to serve any useful purpose. It is, therefore, reasonable to assume that the most appropriate time of application of the hormone herbicides with regard to paddy is the 'early stage' of its growth i.e. about 6 week-old. Alternatively, it may be said that under all circumstances, treatment at the pre-flowering stage of the crop should be avoided.

As a corollary to the above, it may also be suggested that the highest susceptibility of plants to such treatments at the pre-flowering stage can be exploited on the weeds, in case, there exists a remarkable difference in the time of flowering of the weeds and the crop. This will lead to a low rate of seed setting in weed plants and is likely to serve as an effective measure of control of weeds for the subsequent seasons. Such treatments might be particularly useful in case of plantations like sugar-cane, banana, tea, etc.

#### *The comparative performance of the different herbicides :*

Next to the stage of treatment, the selection of a suitable herbicide for the weed control purposes in different crops is very important. The data with regard to paddy in the present observation reveal, that the vegetative growth and reproductive activity are variously affected by the different chemicals.

Thus considering the growth of paddy, it would appear that irrespective of the concentrations applied, the most deleterious effect is produced by the sodium

salt of 2, 4-D and M.C.P.B. The difference between the effects of amine and ester formulations are not very striking.

Considering the effects on the reproductive activity, it has been noted that contrary to the above, there is a greater seed sterility produced by the application of amine and sodium salts of 2, 4-D. (Ref. Graph III).

But the most important aspect of the effect of these chemicals on any crop is the effect on the yield. In the present case, the highest yield has been recorded in the 'control', lower in case of Na-2, 4-D and Ester 2, 4-D and least in case of the M.C.P.B. This suggests that the toxicity of the chemicals in the final reckoning is lowest in case of Na-2, 4-D.

The present observations, therefore, are not in agreement with the observations of Templeman and Halliday (1950) in that, when used at the normal rate, the herbicides do not produce any marked effect on the yield of cereals. These observations also do not conform with the findings of Scragg (1952) that with regard to cereals 2, 4-D was a more potent herbicide than M.C.P.A. It seems that even the cereals vary in their susceptibility to different formulations. From the present observations, it is surmised that the condition of paddy is comparable to that in *Galeopsis tetrahit* and other cereals (Templeman and Wright, 1951) with regard to the susceptibility to M.C.P.B.

It may thus be concluded that as far as possible, the use of M.C.P.A. and M.C.P.B. compounds should be avoided in paddy. Likewise, the study of the comparative susceptibility of other crops to different herbicidal formulations seems necessary before large scale applications of these chemicals are made in agriculture. The necessity of the testing of other compounds on paddy itself is also indicated in order to further reduce the harmful effects of the herbicide even at the early stage of growth.

#### *The relation between the strength of herbicides and the response :*

It has been stated earlier that all the four herbicides produce adverse effects on the growth and yield of paddy. The same holds good for the three strengths (concentrations) of each chemical used in the present experiments, viz., 1,000, 2,500 and 5,000 p.p.m. It will be further noted from Graph I that the reduction in growth is directly related to the concentration. In other words, there is an increasing severity of effect with the concentrations irrespective of the chemicals used and minimum height was recorded at the 5,000 p.p.m. concentration.

Similarly, Graph II suggests that within the range of treatments, the increasing percentage of the seed sterility is the function of concentration. It is, however, interesting to point out that the increase in seed sterility does not take place in the simple ratio of the concentration. That is, the increase of sterility between 2,500 and 5,000 p.p.m. is far greater than the increase from 0 to 1,000 p.p.m. and again from 1,000 to 2,500 p.p.m.

The yield of paddy is also affected by the different concentrations, but unlike seed sterility, the reduction in the yield is proportional to the concentration of the treatment. The above observations lead to the conclusion that a great deal of caution is indicated in selecting the strength of the herbicides, so as to obtain an effective weed control without causing much damage to the crop. Perhaps better results might be obtained by spraying twice with a lower or half of the strength of the herbicide. Further investigations on this line are, therefore, indicated.

#### *Nature and significance of the induced morphological abnormalities :*

The morphological abnormalities caused by the treatment with the hormone herbicides have been described earlier. For convenience, they are being considered as follows :—

(a) *Leaves* : The usual leaf abnormalities produced by the herbicides are epinasty and the stiffness of the flag leaves. Some of the younger leaves appear

curled and do not unfurl normally. However, these abnormalities were infrequent and not severe, except in very high concentrations and did not seem to affect the subsequent performance of the plants.

(b) *Ears* : A number of ear abnormalities have been recorded in the course of present observations. These included the deformities like 'bowed' and 'screwed' ears, short and compact ears and ears lying partially or wholly arrested in the sheath of the flag leaf. Such deformities of the ear have been recorded by Scragg (1952) in wheat, barley and oats. Other abnormalities like paired and whorled spikelets, big glumes and compound grains, frequently observed in herbicide treated wheat, were not found in paddy. However, in a single case (Fig. 6) a 'feathery inflorescence' or ear composed entirely of empty seeds has been recorded.

It is interesting to note that there appears to be a definite relationship between the stage of treatment and the appearance of the ear abnormalities. As a rule there were few abnormalities when the treatment was made at the early stage and none at all at the post-flowering stage. Such abnormalities, on the other hand, were abundant at the pre-flowering stage of treatment. According to Scragg (1952), these abnormalities arise due to a damage caused to the differentiating primordium of the inflorescence. In other words, the development of the ear is affected due to such a treatment. This seems to be a reasonable explanation in the present case also.

However, other abnormalities like 'bowed' and 'screwed' ears may arise only due to the excessive epinasty of the inflorescence axis, while many of other ears fail to emerge from the sheath, partly or wholly, due to the stiffness of the flag leaf, also caused due to hormone herbicide. The author is not aware of any previous record of occurrence of fertile but 'feathery' ear or inflorescence in paddy. It is therefore assumed that the occurrence of such an ear with sterile seeds may be explained on the basis of failure of seed-setting and stiffness of the axis. It is only natural that due to the emptiness of seeds, the ear gives a 'feathery' appearance.

How far the low yield at this stage of treatment is due to morphological abnormalities could not be ascertained. But taking their frequency into consideration, the ear abnormality does not seem to have much effect on the yield of paddy per plot.

(c) *Seeds* : Only two types of seeds were recorded in the present study, viz., "full" and 'empty' seeds. The full seeds were morphologically normal. The origin of the empty seeds may be two-fold :—

(i) due to the failure of formation of the normal gametes.

(ii) due to the developmental failure of the seeds following fertilization.

The former arises due to the upsetting of the nuclear divisions described earlier, while the latter may be caused due to the physiological disbalance caused by an extra supply of auxins. Which of these two causes have played the major rôle in the failure of seed-setting could not, however, be determined from the present experiment.

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# OBSERVATION ON THE NUTRITION OF THE CARP SPAWN OF THE MAHANADI RIVER

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## ABSTRACT

1. Early carp spawn has been reared with zooplankton and various other feed with different combinations of protein, fat, carbohydrate, roughage and vitamins.
2. The record of growth of various species and the total yield has been kept and the value of different type of food has been assessed.
3. Combination of hydrolysed proteins and carbohydrate (50:30) have given the best results while complex proteins do not seem to have been digested well. Pure carbohydrate has given poor results.
4. *Cirrhina reba* has grown well only when fed on zooplankton and seems to depend on digestive enzymes from them as it is unable to digest protein, carbohydrate or fat by itself.
5. Addition of vitamins has generally given better results but further experiments on the rôle of individual vitamins are necessary.
6. Artificial feeding with a mixture of simple proteins and carbohydrate (50:30) may be done to supply food in tanks without natural food.
7. Sorting of *Cirrhina reba* may be easier in tanks without adequate natural food and where fry have been fed as above.

## INTRODUCTION

There is, at present, little knowledge on the nutrition of early carp spawn of Indian major carps like *Catla catla*, *Cirrhina mrigala*, *Labeo rohita* and *Labeo calbasu*. Carp *et al.* (1922, as quoted by Schaeperclaus) found that European carps below 2.5 cms. with yolk sac lived on bosmina, *Anurea cochlearis*, cyclops etc. Lehmann (as quoted by Schaeperclaus) found that carps of only 0.5 cm. length could exist on small cyclops, cypris and chironomus larvae. Alikunhi (1952) found that the survival of the young fry of Indian major carps was 96 to 100 per cent when fed regularly on live zooplankton, but survival and growth were unsatisfactory where planktonic algae were used. Mitra and Mohapatra (1956) found that availability of 0.1 to 0.3 cc. zooplankton per fry gave optimum results in nursery tanks. Schaeperclaus (1933) as translated by Hund has stated that carps have no stomach producing the required pepsin for digestion of proteins, but they have a long intestinal tract, and protein digestion is mainly performed by enzymes developed in the pancreas, which however are less efficient than pepsin especially for proteins of animal origin. He has also said that the artificial food is badly digested by carp-like fish, if 50 per cent, at least, of natural food is not given at the same time.

The present series of experiments were conducted with various artificial feed to compare the results with those of natural nutrition in early spawn of Indian major carps.

## MATERIAL AND METHODS

Two experiments were conducted by rearing early carp spawn 5-7 mm. in length collected from the river Mahanadi in aquaria of a capacity of 15.4 cubic feet. To avoid growth of algae, tubewell water with common salt (25 p.p.m.)



was used and the fry were fed daily with the following feed sprayed as fine powder on the surface of the water.

|                  | Protein      | Fat          | Crude fibre | N. free extract |
|------------------|--------------|--------------|-------------|-----------------|
| Fish meal        | 65-70%       | 5%           | —           | 15-20%          |
| Ledinac          | 50%          | 4%           | —           | 40%             |
| Procasonol       | 50%          | —            | —           | 30%             |
| Rice powder      | 1.09%        | —            | —           | 86.8%           |
| Black gram       | 20.19%       | 4.22         | 9.81        | 62.29%          |
| Rice Bran        | 13.99%       | 20.39        | 14.13       | 35.64%          |
| Mustard oil cake | 36.09%       | 11.11        | 10.05       | 32.76%          |
| Zooplankton      | Not analysed |              |             |                 |
| Starch           | Not analysed |              |             |                 |
| Multivitamin     | Vitamin      | A--2000 I.U. |             |                 |
|                  | "            | B1           | 1 mg.       |                 |
|                  | "            | B2           | 1.2 mg.     |                 |
|                  | "            | B6           | 0.08 mg.    |                 |
|                  | "            | C            | 30 mg.      |                 |
|                  | "            | D            | 200 I.U.    |                 |
|                  | "            | E            | 0.2 mg.     |                 |
|                  | "            | K            | 0.067 mg.   |                 |

Level of water in the aquaria was maintained by addition of tube-well water only. Stocking was done by picking up fry at random from a mixed collection of spawn as these were not identifiable as to species at this stage. Weight has been taken as the criterion of growth, rather than length. Two experiments using spawn collected in different floods were made in order to compare the results, rate of growth, etc. The data relating to the actual observations are given in Tables I (A—C) and II (A—C), at the end, for the two experiments.

#### OBSERVATIONS IN EXPERIMENTS I

In this experiment stocking was done on 21-8-55 and the fry were netted after 8 days when the majority of them could be identified. The mortality varied considerably, the percentage of survival ranging between 38.1 to 98.1. In order to assess the nutritive value of each feed comparable aquaria have been grouped together based on percentage of survival. The observations on each group are given below.

#### EXPERIMENT I—OBSERVATIONS IN GROUP I

The survivals in this group range between 90 and 103 out of an initial stocknig of 105. The yield has been maximum with procasonol (3.26 gm.), next in order being zooplankton (2.63 gm.) and liver (2.28 gm.). These may be considered as good food. Rice powder with an yield of 1.14 gm. fish meal and multivitamin (1.072 gm.) and fish meal only (0.933 gm.) have given average results, while starch (0.309 gm.) black gram (0.594 gm.) rice bran plus multivitamin (0.786 gm.) have given poor results, although the yield is higher than in control (0.175 gm.) where a reduction in weight has resulted from 0.21 gm. initial. It is noticeable that only in case of liver and zooplankton all the specimens grew to a size in 8 days so as to be clearly identifiable. In case of procasonol\* which has given the

\*Procasonol is a hydrolysed protein trade name by Merck-Sharp and Dohme Ltd. comprising of protein carbohydrate concentrate.

Protein (N×62.5) from milk and Soya .. .. 50 G(50%)  
Carbohydrate .. .. 30 G(30%)

Analytical data. . . . Av. total ash—1.5%, sodium chloride 0.12%, calcium hydrogen phosphate  $\text{CaHPO}_4$  0.55%, Magnesium-trace, Potassium-trace fat within 1.5%.

maximum yield, 30 specimens could not be identified, while in case of starch and control none of them could be identified. Analysing the growth of individual species, it is noticed that procasenol has given the best growth in *Catla catla* (0.065 gm.) and *Cirrhina mrigala* (0.068 gm.), liver in *Labeo rohita* (0.066 gm.) and zooplankton in case of *Cirrhina reba* (0.02 gm.). The effect of procasenol on *Labeo rohita* and *Labeo calbasu* could not be studied as these were unrepresented in this group. Adopting the classification as below the comparative value of each feed can be tabulated as follows :

|           |    |    |    |               |     |
|-----------|----|----|----|---------------|-----|
| Very good | .. | .. | .. | 0.065 — 0.15  | gm. |
| Good      | .. | .. | .. | 0.032 — 0.050 | "   |
| Average   | .. | .. | .. | 0.021 — 0.029 | "   |
| Poor      | .. | .. | .. | 0.011 — 0.019 | "   |
| Very poor | .. | .. | .. | below 0.011   |     |

|                             | Catla catla | Labeo rohita | Cirrhina mrigala |
|-----------------------------|-------------|--------------|------------------|
| Fish meal                   | G           | Av           | Av               |
| Liver                       | Av          | V.G.         | G                |
| Rice powder                 | Av          | X            | Av               |
| Zooplankton                 | Av          | G            | G                |
| Rice bran plus multivitamin | P           | X            | P                |
| Fish meal plus multivitamin | G           | X            | G                |
| Black gram                  | P           | P            | P                |
| Procasenol                  | V. G.       | X            | V. G.            |
| Starch                      | X           | X            | X                |

*Labeo calbasu* and *Cirrhina reba* have not been grouped above being slow growing species. Liver and zooplankton have given good growth to the former.

The effect of all artificial feed on *Cirrhina reba* has been unsatisfactory and only zooplankton has given a growth of 0.02 gm. as against the range of 0.003 to 0.008 gm. in other feed. Rice powder seems to have given best result next to zooplankton with a growth of 0.008 gm. average.

#### OBSERVATIONS IN GROUP II

The survival in this group ranges from 75 to 80 out of 105. The maximum yield is 0.79 gm. as against 3.26 gm. in group I. According to standards laid down for group I, the classification of feed may be made as follows :

|                          | Catla catla | Labeo rohita | Cirrhina mrigala |
|--------------------------|-------------|--------------|------------------|
| Starch plus multivitamin | X           | X            | X                |
| Rice bran                | Av          | P            | Av               |
| Mustard oil cake         | G           | X            | G                |

Table showing the comparative growth of carp spawn in Exp. I and II

|                                 | Total<br>no.<br>survi-<br>ved | Total<br>wt.<br>in<br>gms. | Catla |          |            | L. rohita |          |            | C. mrigala |          |            |
|---------------------------------|-------------------------------|----------------------------|-------|----------|------------|-----------|----------|------------|------------|----------|------------|
|                                 |                               |                            | no.   | Av.<br>L | Av.<br>Wt. | no.       | Av.<br>L | Av.<br>Wt. | no.        | Av.<br>L | Av.<br>Wt. |
| 8 days growth in<br>procasenol  | I 90                          | 3.26                       | 31    | 1.76     | 0.065      | —         | —        | —          | 14         | 1.7      | 0.068      |
|                                 | II 49                         | 0.925                      | 5     | 1.5      | 0.06       | 7         | 1.66     | 0.068      | 3          | 1.51     | 0.048      |
| 8 days growth in<br>fish meal   | I 90                          | 0.933                      | 5     | 1.41     | 0.035      | 7         | 1.35     | 0.029      | 10         | 1.28     | 0.025      |
|                                 | II 40                         | 0.825                      | 5     | 1.51     | 0.051      | 7         | 1.64     | 0.06       | 3          | 1.51     | 0.05       |
| 8 days growth in<br>fresh liver | I 100                         | 2.28                       | 25    | 1.63     | 0.025      | 5         | 1.75     | 0.066      | 20         | 1.55     | 0.038      |
|                                 | II 82                         | 3.203                      | 28    | 1.46     | 0.037      | 5         | 1.24     | 0.02       | 40         | 1.51     | 0.065      |

## OBSERVATIONS IN GROUP III

In this group growth was very poor in both *Labeo calbasu* and *Cirrhina reba*. The survival in this group ranges between 57 and 68 out of 105 and the relative value of the feed is tabulated below.

|                              | Catla catla | Labeo rohita | Cirrhina mrigala |
|------------------------------|-------------|--------------|------------------|
| Rice powder plus ANDEC       | G           | X            | Av               |
| Ledinac                      | Av          | G            | G                |
| Black Gram plus multivitamin | P           | P            | P                |
| Rice powder                  | P           | P            | P                |

## EXPERIMENT II

The experiment was done with Mahanadi spawn collected at Nuapatna on 30-8-56 and the period of rearing was two weeks against one week in experiment I. The average mortality has been found to be higher in this case which may be due to the spawn being from second flood, damaged in transit or other causes. In order to have comparative data on growth in 8 days, fish out of three aquaria where growth was perceptible were removed and the relative merits of the various feed are indicated below for this group only.

Considering the relative merits according to standards laid down the results were :

|             | Catla catla | Labeo rohita | Cirrhina mrigala |
|-------------|-------------|--------------|------------------|
| Procasanol  | VG          | VG           | G                |
| Fish meal   | G           | VG           | G                |
| Fresh liver | G           | Av           | VG               |

As the survivals are not comparable it is not possible to judge the relative growth of the various species in the two experiments. In general all the three types of food have given good results and growth has been comparable.

In the second series of experiments with a rearing period of 14 days three groupings have been made according to survival. In the first group the survival ranged between 82 and 97 out of 105, the highest total yield being from rice powder and ABDEC (3.343 gm.), Ledinac (3.19 gm.) and Black grams with multivitamin (1.716 gm.). The effect of various feeds is tabulated below.

|                             | Catla catla | Labeo rohita | Cirrhina mrigala |
|-----------------------------|-------------|--------------|------------------|
| Ledinac                     | G           | G            | G                |
| Rice powder and ABDEC       | G           | G            | G                |
| Black gram and multivitamin | Av          | G            | G                |

*Cirrhina reba* showed poor growth with rice powder and ABDEC and Black gram and multivitamin.

In the second group 64-72 fish survived out of 105. The yield was 2.208 gm. with zooplankton, as against 3.3 gm. with rice powder and ABDEC in group I. The relative effect of the various feeds is tabulated below.

|             | <i>Catla catla</i> | <i>Labeo rohita</i> | <i>Cirrhina mrigala</i> |
|-------------|--------------------|---------------------|-------------------------|
| Zooplankton | Av                 | G                   | X                       |
| Rice bran   | Av                 | Av                  | P                       |
| Rice powder | Av                 | G                   | Av                      |
| Black gram  | Av                 | Av                  | G                       |

In group III, the survival fluctuated from 19-58 out of 105 and the maximum total yield was only 1.65 gms. with fish meal and multivitamins. The relative value of each food is tabulated below.

|                             | <i>Catla catla</i> | <i>Labeo rohita</i> | <i>Cirrhina mrigala</i> |
|-----------------------------|--------------------|---------------------|-------------------------|
| Starch and multivitamin     | X                  | X                   | X                       |
| Black gram and multivitamin | G                  | Av                  | G                       |
| Rice and multivitamin       | G                  | Av                  | G                       |
| Mustard oil cake            | VG                 | Av                  | VG                      |
| Fish meal and multivitamin  | VG                 | G                   | VG                      |
| Starch                      | P                  | X                   | P                       |

In general it is seen that fish meal has given good result with *Catla catla* and with vitamins the results have been very good. In *Labeo rohita* the result varies from average to very good with or without vitamins. In *Cirrhina mrigala* the result is average to good and very good with vitamins. Fresh liver has given average to good result with *Catla catla*, average to very good result with *Labeo rohita*, and good to very good with *Cirrhina mrigala*. Zooplankton has given similar result as liver i.e. average with *Catla catla*, very good with *Labeo rohita* and good with *Cirrhina mrigala*. Procasenol has given very good result in all cases. Black gram has yielded poor to average result in *Catla catla*, *Labeo rohita* and poor to good result in *Cirrhina mrigala*. Black gram with vitamins has given average result with *Catla catla* and *Cirrhina mrigala*. Mustard oil cake which is commonly used as fish food has given very good result with *Catla catla* and *Cirrhina mrigala* and average result with *Labeo rohita*. Rice powder without vitamin has given poor to average result with *Catla catla* and *Cirrhina mrigala*, good result with *Labeo rohita*, with ABDEC, the result has been good in all cases. In case of rice bran without vitamins the result is average in *Catla catla* and *Labeo rohita*, poor in *Cirrhina mrigala*. With multivitamin the results vary from good to poor in *Catla catla* and *Cirrhina mrigala*, average in *Labeo rohita*; starch with or without vitamin has given uniformly poor results. Procasenol, fresh liver and zooplankton may be said to be fattening food as shown in experiment I and Ledinac and rice powder with ABDEC in experiment II.

Variation in growth under different feeds has been considerable as indicated below.

*Average weight in grams*

|                         | Minimum | Maximum |
|-------------------------|---------|---------|
| <i>Catla catla</i>      | 0.011   | 0.065   |
| <i>Labeo rohita</i>     | 0.018   | 0.066   |
| <i>Cirrhina mrigala</i> | 0.011   | 0.068   |
| <i>Labeo calbasu</i>    | 0.004   | 0.021   |
| <i>Cirrhina reba</i>    | 0.003   | 0.02    |
| <i>Catla catla</i>      | 0.01    | 0.15    |
| <i>Labeo rohita</i>     | 0.02    | 0.045   |
| <i>Cirrhina mrigala</i> | 0.01    | 0.065   |
| <i>Labeo calbasu</i>    | 0.01    | 0.042   |
| <i>Cirrhina reba</i>    | 0.003   | 0.021   |

### DISCUSSION

Comparatively very little work has been done on the nutritional requirements of the Indian carp spawn at a very early stage, although detailed study has been made abroad in culturable fishes, particularly trout. According to compilation of results by Hall (1949) 2.06 to 3.29 lbs. of beef, liver 70 per cent and salmon egg meal 30 per cent are required to produce 1 lb. of rainbow and brook trout. Proteins do not take the first place in food; excess carbohydrates interfere in the digestion; and fish for quick growth do not require roughage. Alikunhi (1952) considers zooplankton adequate for proper nutrition of early carp spawn.

The present experiments show appreciable growth of carp spawn at a stage of 5 to 7 mm. just after absorption of the yolk sac on a mixed diet of protein and carbohydrate only without any combinations of natural food.

It may be presumed that the digestive juices have been able to digest a substantial part as is indicated by the following figures:

*Quantity of food required to produce one gram of fish*

|             |    |    |    |           |
|-------------|----|----|----|-----------|
| Procasenol  | .. | .. | .. | 3.6 gms.  |
| Zooplankton | .. | .. | .. | 4.5 gms.  |
| Liver       | .. | .. | .. | 5.7 gms.  |
| Rice powder | .. | .. | .. | 10.4 gms. |
| Fish meal   | .. | .. | .. | 11.2 gms. |

Procasenol and liver, however, have given better results than zooplankton, but if it is considered that both procasenol and liver are concentrated while zooplankton contains considerable water it is difficult to say to what extent the artificial food is superior to zooplankton. It is, however, clearly shown that early carp spawn in the absence of zooplankton obtain good nutrition from proteins mixed with carbohydrate. This is of practical importance in rearing carp spawn in tanks where natural food could not be grown.

Generally protein-rich diets have produced better results, while starch by itself has not been digested. Presumably the assimilation capacity for starch is poor. In case of proteins, hydrolysed proteins have been better absorbed. Although the rôle of each kind of vitamin has not been studied in these experiments, addition of multivitamins has yielded comparatively better results. Zobairi



## IA

*Observation in Group I)*

| C. mrigala |      |      | C. reba |      |       | Unidentified |      |       | Total no. of survi- | Total yield in gms. | Total feed in gms. | Period of rear- ing |
|------------|------|------|---------|------|-------|--------------|------|-------|---------------------|---------------------|--------------------|---------------------|
| No.        | A.L. | A.W. | No.     | A.L. | A.W.  | No.          | A.L. | A.W.  | val                 |                     |                    |                     |
| 10         | 1.28 | .025 | 19      | 0.86 | .003  | 27           | .8   | .004  | 90                  | .933                | 12                 | 8 days              |
| 20         | 1.55 | .038 | 27      | 1.0  | .0037 | —            | —    | —     | 100                 | 2.28                | —                  | —                   |
| 18         | 1.3  | .025 | 15      | 0.74 | .008  | 15           | .75  | .004  | 99                  | 1.14                | —                  | —                   |
| 19         | 1.4  | .037 | 47      | 1.59 | .02   | —            | —    | —     | 99                  | 2.63                | —                  | —                   |
| 20         | 1.19 | .011 | 28      | .73  | .003  | 31           | .85  | .004  | 91                  | 0.786               | —                  | —                   |
| 12         | 1.33 | .03  | 39      | .88  | .003  | 25           | .8   | .0036 | 91                  | 1.072               | —                  | —                   |
| 11         | 1.33 | .03  | 20      | .74  | .003  | 36           | .6   | .003  | 90                  | 0.594               | —                  | —                   |
| 14         | 1.7  | .068 | 15      | 1.09 | .004  | 30           | .7   | .004  | 90                  | 3.26                | —                  | —                   |
| —          | —    | —    | 6       | .7   | .003  | 97           | .7   | .003  | 103                 | 0.309               | —                  | —                   |
| —          | —    | —    | —       | —    | —     | 58           | .74  | .003  | 58                  | 0.175               | —                  | —                   |

A.L. in cms.—Average Length in cms.

A.W. in gms.—Average weight in gms.

Initial stocking : No. 105

Size—5-7 mm.

Av. Wt.—.002 gms.

Total weight : .21 gms.

## Ib

*Observations in Group II)*

| C. mrigala |                    |                    | C. reba |                    |                    | Unidentified |                    |                    | Total no. of survi- | Total yield in gms. | Total feed in gms. | Period of rear- ch |
|------------|--------------------|--------------------|---------|--------------------|--------------------|--------------|--------------------|--------------------|---------------------|---------------------|--------------------|--------------------|
| No.        | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.     | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.          | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | val                 |                     |                    |                    |
| —          | —                  | —                  | 19      | 0.71               | 0.0031             | 61           | 0.7                | 0.003              | 80                  | 0.243               | 12                 | 8 days             |
| 8          | 1.39               | 0.021              | 15      | 0.73               | 0.003              | 19           | 0.7                | 0.003              | 75                  | 0.714               | —                  | —                  |
| 9          | 1.56               | 0.037              | 27      | 0.75               | 0.0037             | 30           | 0.7                | 0.003              | 75                  | 0.79                | —                  | —                  |

## Ic

*Observations in group III)*

| C. mrigala |                    |                    | C. reba |                    |                    | Unidentified |                    |                    | Total no. of survi- | Total feed in gms. | Total yield in gms. | Period of rear- ing |
|------------|--------------------|--------------------|---------|--------------------|--------------------|--------------|--------------------|--------------------|---------------------|--------------------|---------------------|---------------------|
| No.        | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.     | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.          | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | val                 |                    |                     |                     |
| 13         | 1.25               | 0.021              | 18      | 0.77               | 0.003              | 27           | 0.75               | 0.003              | 68                  | 0.741              | 12                  | 8 days              |
| 16         | 1.42               | 0.032              | 3       | 1.11               | 0.006              | 23           | 0.7                | 0.003              | 67                  | 1.32               | —                   | —                   |
| 34         | 1.19               | 0.018              | 9       | 0.8                | 0.004              | 16           | 0.7                | 0.003              | 64                  | 0.581              | —                   | —                   |
| 10         | 1.26               | 0.021              | 2       | 0.8                | 0.003              | 28           | 0.7                | 0.0037             | 57                  | 0.559              | —                   | —                   |
| —          | —                  | —                  | —       | —                  | —                  | 58           | 0.74               | 0.003              | 58                  | 0.175              | No feed             | —                   |



TABLE  
(Experiment II,

| Nature of feed                 | C. Catla |                    |                    | L. rohita |                    |                    | L. calbasu |                    |                    |
|--------------------------------|----------|--------------------|--------------------|-----------|--------------------|--------------------|------------|--------------------|--------------------|
|                                | No.      | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.       | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.        | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. |
| Lodmae                         | 38       | 1.35               | 0.033              | 24        | 1.34               | 0.034              | 2          | 1.45               | 0.042              |
| Rice powder ABDEC              | 34       | 1.65               | 0.0528             | 10        | 1.42               | 0.0325             | —          | —                  | —                  |
| Black gram and<br>Multivitamin | 37       | 1.5                | 0.02               | 1         | 1.6                | 0.03               | —          | —                  | —                  |

TABLE  
(Experiment II,

| Nature of feed | C. Catla |                    |                    | L. rohita |                    |                    | L. calbasu |                    |                    |
|----------------|----------|--------------------|--------------------|-----------|--------------------|--------------------|------------|--------------------|--------------------|
|                | No.      | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.       | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.        | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. |
| Zooplankton    | 36       | 1.33               | 0.023              | 15        | 1.53               | 0.05               | 21         | 1.1                | 0.03               |
| Rice bran      | 23       | 1.3                | 0.022              | 3         | 1.28               | 0.028              | 3          | 0.8                | 0.0105             |
| Rice powder    | 27       | 1.36               | 0.025              | 6         | 1.33               | 0.03               | 3          | 0.95               | 0.005              |
| Black gram     | 32       | 1.47               | 0.025              | 4         | 1.32               | 0.029              | 3          | 0.95               | 0.004              |

TABLE  
(Experiment II,

| Nature of feed                  | C. Catla |                    |                    | L. rohita |                    |                    | L. calbasu |                    |                    |
|---------------------------------|----------|--------------------|--------------------|-----------|--------------------|--------------------|------------|--------------------|--------------------|
|                                 | No.      | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.       | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. | No.        | A.L.<br>in<br>cms. | A.W.<br>in<br>gms. |
| Starch and<br>multivitamin      | —        | —                  | —                  | —         | —                  | —                  | —          | —                  | —                  |
| Pulsepowder and<br>multivitamin | 16       | 1.24               | 0.03               | 5         | 1.38               | 0.023              | —          | —                  | —                  |
| Rice bran and<br>multivitamin   | 11       | 1.57               | 0.036              | 7         | 1.54               | 0.022              | 1          | 1.2                | 0.023              |
| Control                         | 5        | 1.12               | 0.01               | —         | —                  | —                  | —          | —                  | —                  |
| Mustard oil cake                | 7        | 1.91               | 0.08               | 5         | 1.4                | 0.023              | —          | —                  | —                  |
| Fish meal and<br>multivitamin   | 3        | 2.24               | 0.15               | 8         | 1.6                | 0.045              | 1          | 1.2                | 0.03               |
| Starch                          | 5        | 1.02               | 0.012              | —         | —                  | —                  | —          | —                  | —                  |

## IIa

*Observations in group I)*

| C. mrigala |              |              | C. reba |              |              | Unidentified |              |              | Total no. of survi- | Total yield in gms. | Total feed in gms. | Period of rearing |
|------------|--------------|--------------|---------|--------------|--------------|--------------|--------------|--------------|---------------------|---------------------|--------------------|-------------------|
| No.        | A.L. in cms. | A.W. in gms. | No.     | A.L. in cms. | A.W. in gms. | No.          | A.L. in cms. | A.W. in gms. | val                 |                     |                    |                   |
| 31         | 1.34         | 0.032        | 2       | 1.0          | 0.023        | —            | —            | —            | 97                  | 3.19                | 21.0               | 14 days           |
| 31         | 1.5          | 0.035        | 9       | 0.36         | 0.015        | 3            | 0.7          | 0.003        | 87                  | 3.343               | —                  | „                 |
| 20         | 1.5          | 0.04         | 13      | 0.9          | 0.007        | 22           | 0.72         | 0.003        | 82                  | 1.716               | —                  | „                 |

## IIb

*Observations in group II)*

| C. mrigala |              |                | C. reba |              |                | Unidentified |              |              | Total no. of survi- | Total yield in gms. | Total feed in gms. | Period of rearing (days) |
|------------|--------------|----------------|---------|--------------|----------------|--------------|--------------|--------------|---------------------|---------------------|--------------------|--------------------------|
| No.        | A.L. in cms. | A.W. in grams. | No.     | A.L. in cms. | A.W. in grams. | No.          | A.L. in cms. | A.W. in gms. | val                 |                     |                    |                          |
| —          | —            | —              | —       | —            | —              | —            | —            | —            | 72                  | 2.208               | 21.0               | 14                       |
| 10         | 1.18         | 0.0115         | —       | —            | —              | 29           | 0.7          | 0.003        | 68                  | 0.955               | —                  | 14                       |
| 11         | 1.37         | 0.023          | 4       | 1.17         | 0.021          | 15           | 0.7          | 0.003        | 66                  | 0.99                | —                  | 14                       |
| 22         | 1.62         | 0.032          | 2       | 1.2          | 0.018          | —            | —            | —            | 64                  | 1.668               | —                  | 14                       |

## IIc

*Observations in Group III)*

| C. mrigala |              |              | C. reba |             |              | Unidentified. |              |              | Total no. of survi- | Total yield in gms. | Total feed in gms. | Period of rearing. |
|------------|--------------|--------------|---------|-------------|--------------|---------------|--------------|--------------|---------------------|---------------------|--------------------|--------------------|
| No.        | A.L. in cms. | A.W. in gms. | No.     | A.L. in cm. | A.W. in gms. | No.           | A.L. in cms. | A.W. in gms. | val                 |                     |                    | (days)             |
| —          | —            | —            | 5       | 0.91        | 0.008        | 59            | 0.85         | 0.005        | 58                  | 0.201               | 21                 | 14                 |
| 7          | 1.25         | 0.0307       | 5       | 1.08        | 0.019        | 24            | 0.7          | 0.003        | 57                  | 1.01                | 21                 | 14                 |
| 8          | 1.85         | 0.04         | 9       | 1.17        | 0.012        | —             | —            | —            | 36                  | 0.745               | 21                 | 14                 |
| 3          | 1.1          | 0.01         | —       | —           | —            | 28            | 0.75         | 0.002        | 36                  | 0.108               | N.f.               | 14                 |
| 4          | 1.72         | 0.065        | 2       | 1.3         | 0.021        | 10            | 0.7          | 0.003        | 28                  | 1.152               | 21                 | 14                 |
| 4          | 2.02         | 0.12         | 7       | 0.91        | 0.01         | 11            | 0.75         | 0.002        | 25                  | 1.65                | „                  | 14                 |
| 3          | 1.03         | 0.011        | 5       | 0.84        | 0.003        | 6             | 0.7          | 0.003        | 19                  | 0.126               | „                  | 14                 |

N.f.—Not fed,

(1956) has noted that absence of vitamin B<sub>12</sub> causes disintegration of fin membranes and loss of equilibrium in *Cyprinus carpio* after about 180 days of rearing with pea nut and fish meal (10 : 1). No abnormal condition has been noted in aquaria without vitamins within the short rearing period of 14 days. It is likely that the period of rearing was not sufficient to make the deficiencies manifest.

Survival in aquaria has been very high in the first flood fry and the findings are in keeping with the results of previous authors (Alikunhi, 1952). There is general agreement that while lack of nutrition affects growth it does not lead to mortality unless there is complete lack of it and the cause for mortality has to be traced to unfavourable physico-chemical conditions and predators, the latter being the more important factor. Mortality is also caused by disease e.g. fungus in fry, injured in transit or otherwise. The variation in mortality in the aquaria is considerable and is accounted for by injury in handling at the time of stocking as the fry was counted individually. By taking observations on the groupings made according to survival the availability of food is kept constant in a particular group and error has been minimised.

The digestive capacity of protein by *Cirrhina reba* seems to be different from that of the major carp spawn as it has shown very poor growth under all types of artificial food used, but growth is good in case of zooplankton. *Cirrhina reba* is the main unwanted variety mixed with the spawn of major carps and it may be of practical value to feed mixed spawn with suitable artificial feed only, instead of zooplankton, to facilitate mechanical sorting.

The specially significant results given by procasenol suggest that a suitable cheap food mixture should consist of simple proteins and carbohydrate in a proportion of 50 : 30 without roughage. Further experiments are necessary on the part played by individual proteins in the nutrition of early spawn. The results have applicability in nurseries where growth of natural food is poor, or manuring etc. have not been possible.

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# MECHANISM OF RESISTANCE OF PADDY (*ORYZA SATIVA* L.) TO *PIRICULARIA ORYZAE* CAV.

## I. GENERAL CONSIDERATIONS

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### ABSTRACT

The importance of nyctotemperature in the resistance/susceptibility relationship of paddy to blast is discussed in relation to the nitrogen metabolism of the host. The probable significance of cuticular excretions in modifying the physico-chemical nature of the leaf blade and in stimulating/inhibiting spore germination at the infection court or in promoting microbial antagonism in the phyllosphere has also been indicated.

### INTRODUCTION

A critical consideration of the previous literature regarding the infectivity and spread of *Piricularia oryzae* on rice plants reveals that low temperatures (24 to 28°C.) and high humidities (above 90 per cent) favour infection (Anderson *et al.*, 1947). In the field, incidence of disease is maximum in crops sown in October when these conditions are more or less fulfilled (Thomas, 1940). Hashioka (1943*b*) however considered temperature to be more important than humidity for successful infection. It has also been observed that resistance increases with rise in air and soil temperatures (Hashioka, 1943*a*) as well as with the age of the host (Hashioka, 1943*c*). Pathogenicity seems to be lower in the booting than in the seedling stage (Abe, 1936) and in the same plant susceptibility appears to be greatest in the middle leaves and on spikelets (Abe, 1937). Imura (1938) noted that while initial infection by *P. oryzae* is at an optimum with slight shading, lesions enlarge later under greater light intensity.

Another aspect of the problem is that of the influence of nitrogenous fertilizers on susceptibility. That increased application of nitrogenous fertilizers increased the disease proneness of susceptible types but had little or no effect on resistant types has been confirmed by many workers (Thomas, 1938 : Sawada, 1940 : Krishnaswami, 1952). Hashioka (1944*a, b*) considered that the relation between resistance and the environmental temperature has to be ascribed mainly to the nitrogen metabolism of the host as this influence of fertilizers on resistance is stronger at lower temperatures. He concluded that in general the resistance-susceptibility relation is more strongly influenced by temperature than by fertilizing. Otani (1952) observed that the amount of soluble nitrogenous components is apparently connected with susceptibility and there existed no relation between sugar content and susceptibility. Aspartic acid was found to be low at the season of maximum susceptibility to *P. oryzae* (Tanaka and Katsuki, 1952).

In spite of the increasing emphasis laid on root excretions and their impact on the root surface and rhizosphere microfloras in relation to soil-borne fungal diseases, an analogous situation arising at the leaf surface by way of cuticular excretions is rather little recognized though Brown, as early as 1922, indicated that organic metabolites and inorganic ions diffusing out of the cell interior to the exterior

of the epidermis stimulated the germination of conidia of *Botrytis cinerea*. In their recent report, Kovács and Szeőke (1956) have indicated the inhibitory/stimulatory influence of cuticular excretions (by exosmosis) on the germination of spores of *Botrytis cinerea*, *Puccinia tritici* and other fungi. Hafiz (1952) surmised that the greater secretion of malic acid by gram (*Cicer arietinum*), resistant to *Mycosphaerella* blight was detrimental to spore germination and germ tube growth. The fungistatic properties of leaf exudates were demonstrated by Topps and Wain (1957). Recently, in this laboratory, chromatographic analysis of leaf excretions of paddy, resistant and susceptible to *P. oryzae*, has shown certain differences in their amino acid and organic acid compositions (Subba Rao and Suryanarayanan, 1957).

That organic and inorganic nutrients are indeed lost from plant foliage by leaching has been firmly established by Long *et al.* (1956) in their radioisotope studies. This loss has been observed to occur throughout the growing season, with the quantities increasing greatly just before maturity and death of the foliage, the rate of loss being influenced by a variety of factors as light, nutrient level, maturation of the foliage, etc. Large quantities of reducing substance(s) and ninhydrin-positive materials suggesting a polypeptide in the original samples have been found in the leachates. Losses of K, Ca, N and P were also recorded.

In view of the foregoing considerations on cuticular excretions, it then becomes obvious that the phenomenon of microbial antagonism assumes an equal importance at the leaf surface as it does at the remoter regions of the plant, viz., the root and in the rhizosphere. Comparatively little work has been reported on the interrelationships, be it synergistic, competitive or antagonistic, of phyllosphere microflora in relation to an airborne infection like the blast of paddy though Hemmi *et al.* (1936) found that admixture of conidia of *P. oryzae* and *Ophiobolus miyabeanus* in the inoculum reduced their pathogenicity to rice. Similarly, Kitani (1941) recorded a yellow, gram-positive bacterium often associated with *P. oryzae* tending to reduce the occurrence of the disease, but not the size of the leaf spot. Even though the phenomenon of antagonism is not likely to be so pronounced on the leaf surface as it is believed to be in the rhizosphere, it is, nevertheless, a case in point at least in the earlier stages of growth of a pathogen on the intact foliar surface. It is, therefore, reasonable to assume that the nature and the amount of substances present in the cuticular excretions might as well play a rôle, though in an indirect and lesser measure, in the disease proneness of the host.

Besides the above, it is relevant to mention here that the blast disease of rice occurs under this laboratory conditions as well as in the field only during the winter months, December and January and that the author has noticed in the past seven years that more frequently than not, infection frequently fails to establish on susceptible plants raised under ordinary green house conditions even during the colder months with adequate humidity. Similarly, the incidence of the disease in the field has been observed to fluctuate widely from year to year. It then needs hardly any emphasis that the prime requisite for a careful evaluation of the problem on hand is one of examining the factors governing susceptibility rather than resistance. In other words, in the last analysis, we might expect that an understanding of the biochemical mechanisms at play in the phenomena of resistance and susceptibility would take nearer the solution than an intimate analysis of the biochemical constituents of the genetically resistant and susceptible types. The attempts made in this direction are described below.

## METHODS

### *Temperature and humidity control*

In the earlier experiments the desired temperature of 24 to 26°C and 100 per cent humidity were achieved in a well insulated incubator by evaporative

cooling of water by means of compressed air. For lower temperatures below 24°C a thermostatically controlled refrigerating system was used.

#### *Chromatographic techniques*

Both circular paper chromatography as standardized in this laboratory and the micro-ascending technique of Rockland and Dunn (1949) were used.

#### *Sporulation of the pathogen*

Obtaining spores in sufficient quanta for artificial inoculation studies is another difficulty that is usually met with in this problem. Although previous studies in this laboratory have indicated that the fungus sporulates normally on 1 per cent potato-dextrose agar (Apparao, 1956), the intensity of sporulation depends again on the temperature of incubation. It has also been observed that old cultures maintained on potato-dextrose agar become progressively non-pathogenic and even fail to grow on sterilized straw bits while fresh isolates grow readily on them and sporulate profusely (Suryanarayanan, 1955). In the present study, fresh isolates of *P. oryzae* were therefore made and stock cultures maintained on 1 per cent potato-dextrose agar. Initially spores were obtained from sterilized straw cultures but by the third subculturing the fungus failed to grow over this substrate and now this apparent failure of the fungus to grow on stem bits is ascribed to the augmented environmental temperature, for, such cultures when incubated at 24 to 26°C readily colonized the straw bits and sporulated. In the course of these investigations it was also observed that colonies developed from spores produced spores in greater quantity than those developed from sterile mycelial bits. So the method of injecting a drop of spore suspension with the help of a sterile hypodermic syringe was resorted to while subculturing the new isolates in preference to the conventional method of inoculating with bits of agar.

#### *Methods of obtaining spore suspension*

In studies of spore germination it is apparent that a suspension of spores free from mycelial bits should be obtained. The usual practice of adding water and vigorously shaking the cultures or detaching the conidia with a platinum loop always resulted in an admixture of conidia and mycelial bits. Differential centrifugation was not useful in this direction. A method had to be sought in which conidia alone would be detached and the use of a synthetic detergent like 'Tween-20' (tris (polyoxyethylene) sorbitan monolaurate) was found to fulfil the purpose. Concurrently the toxicity of 'Tween' to *Piricularia* spores was also evaluated and a concentration below 0.05 per cent was satisfactory. Washing spores free of adhering metabolites could be achieved only when a 0.9 per cent saline was used, for, centrifuging a water suspension of *Piricularia* spores even at 7,000 r.p.m. in an International Centrifuge did not effect sedimentation.

#### *The physico-chemical properties of the leaf blades of rice*

Inasmuch as the surface of the leaf blades of rice is highly hydrophobic, it is impossible to place a drop of aqueous spore suspension over the leaf. On the contrary an aqueous suspension containing 0.05 per cent 'Tween' not only greatly increased the wettability of the surface but also promoted the appressorial formation to a certain extent both on the leaf surface and on glass slides, possibly due to the contact stimulus brought about by the lowering of surface and interfacial tensions by the surface active agent. It is known that additives like amino acids, peptides and proteins sometimes improve detergency. Here then is a fragment of evidence that the physico-chemical phenomena playing at leaf surfaces might as well be influenced by such substances present in cuticular excretions.

### *Spore germination studies*

The *in vitro* studies on germination were conducted on microscope slides in Petri dishes providing optimum humidity. In the *in vivo* studies, however (germination of spores on the leaf surface), the usual method of clearing the leaf with pyridine and staining with lactophenol-cotton blue was found to be not satisfactory for paddy leaves. Apart from the poor clarity of the preparations the ungerminated spores were lost during the process of clearing. To obviate this difficulty a other technique was evolved. A piece of cellulose adhesive tape was pressed over the leaf and peeled off. The adhesive tape removed both the germinated and ungerminated spores with an impression of the epidermal layer, facilitating a quick and direct examination of a large number of preparations under the microscope.

### *Detached leaf cultures*

Since infection frequently failed to establish itself on leaves attached to the plant and as the physiological age of the individual leaves varied with their relative positions on the plant, a recourse was taken to detached leaves on account of the ease with which they could be manipulated under more controlled conditions. Detached leaves when used were either stood on water or appropriate nutrient solutions or floated in Petri dishes containing the respective solutions.

### *Strains of paddy used*

Strains of paddy evolved by the Madras State Department of Agriculture, viz., CO<sub>4</sub>,  $\times$  6522 (Resistant) and ADT 10 and CO 13 (Susceptible) to *Piricularia* were used of which CO 4 and ADT 10 are long duration types and  $\times$  6522 and CO 13 are short duration types.

## EXPERIMENTAL

### 1. *Determining the age of susceptibility*

Repeated inoculation on both resistant and susceptible types at different stages of growth, viz., from seedling to flowering generally failed to establish infection though during December few spots developed on one month old CO 13 seedlings. Infection however failed to establish at the same age level when the experiment was repeated in February. It must be stated here that these plants were grown under ordinary green house conditions but the plants were always incubated at 24 to 26°C with 100 per cent humidity after inoculation.

The failure of the fungus to infect even susceptible types was naturally sought in the ability of the spores to germinate under these conditions. No conclusive evidence could be obtained since spores sometimes germinated equally well on either the susceptible or resistant types and at other times they did not germinate on both of them. Exudates collected from the resistant and susceptible leaf surfaces showed no significant difference either in the percentage germination or germ tube length.

### 2. *Studies on detached leaves*

As considerable variation occurred on leaves attached to the plant, further experiments were carried out on detached leaves. Comparable leaves of resistant and susceptible types from different positions of the plant floated/stood on water, different sugar solutions, nitrogen sources, organic acids, ammonium salts of organic acids, amino acids, trace element solutions, vitamin solutions,  $\beta$ -indolyl acetic acid and  $\beta$ -indolyl butyric acid were without effect on the production of typical lesions even though these cultures were given the optimum temperature (24

to 26°C and 100 per cent humidity. Neither light nor darkness had any effect under these conditions. It is again relevant to mention here that the samples of leaves were from plants grown under ordinary green house conditions. Nothing conclusive could be gained by spore germination studies with these treatments. Nevertheless, it is of interest to mention here that on detached leaves kept on 10 per cent sucrose solutions and 0.1 M ammonium acetate a number of other fungi belonging to the genera, *Fusarium*, *Nigrospora*, *Curvularia*, *Helminthosporium*, *Mucor* and other unidentified fungi developed. This phenomenon, however, did not occur on water controls or on leaves stood in solutions of other organic ammonium salts.

#### *Nitrogen nutrition of the host and disease incidence*

Even though increased application of nitrogen has been reported to augment the susceptibility of the host, such a treatment in the present study did not result in rendering the CO 13 plants susceptible to the disease.

#### *Effect of photoperiods*

Subjecting the susceptible plants to different photoperiods in a chamber specially constructed in this laboratory, under varied nutrient conditions did not affect disease proneness of the host to any extent. At one time, when infected plants were however exposed to a greater light intensity the lesions enlarged more rapidly than under the normal diffuse light of the green house. Under these conditions, when ammonium sulphate was also added to the soil, so much glutamine (identified chromatographically) was synthesized that they crystallized at the leaf tips. In resistant varieties, however, this synthesis of glutamine appeared to be less marked.

#### 5. *Effect of foliar sprays on infection*

With a view to find out if nutrient sprays of nitrogenous salts would be more efficient in rendering the CO 13 plants susceptible to the disease than the application of nitrogen to the soil, urea and ammonium nitrate were sprayed on leaves for a week at 0.1 M concentration. In addition, the plants were also subjected to different photoperiods. But these treatments did not facilitate infection. Similarly, adenine sprays under the same conditions proved not useful in promoting infection. Leaves detached from such sprayed plants and placed in sugar or organic acid solutions under light to facilitate the synthesis of amides did not favour infection.

#### 6. *Effect of thermoperiods*

The importance of growing plants under controlled environmental conditions for precise experimentation has been increasingly stressed (Went, 1957). Because of the influence of nyctotemperatures on the general physiology of the plant, especially in the translocation of sugar and consequently on the nitrogen metabolism, it was thought worthwhile to subject the susceptible CO 13 plants to lower night temperatures (20°C) than that obtained in the green house. This experiment was performed in the month of February on one month old CO 13 paddy seedlings and when the air temperature touched a maximum of 28°C and a minimum of 24°C in the green house. The thermoperiodic treatment was continued for a week after which they were inoculated with *P. oryzae*. Plants of the same age growing under the normal day-night temperatures were also inoculated simultaneously. The results indicated that while the plants subjected to the thermoperiodic treatment took infection on the still developing younger leaves, no infection occurred on any leaves on the plants that were left over in the green house during nights.



## DISCUSSION

Even though no clear cut evidences are forthcoming from the foregoing experiments it stands out to reason that the failure of infection on susceptible types grown under ordinary green house conditions and in leaves detached from such plants and floating in organic and inorganic solutions even at the optimum temperature and humidity is mainly reflected in the altered host metabolism. Of considerable interest is the observation that in one month old CO 13 plants, given a low nyctotemperature (20°C), the young and still developing leaves took up infection while comparable plants with higher night temperatures were not affected. It is perhaps pertinent at this point to enquire how the nyctotemperature has altered the host metabolism. It has been observed that at high nyctotemperatures the nitrate content of tomato leaves is very high owing to the low nitrate reduction and that the plants become obviously nitrogen deficient (Went, 1957). Van Gundy and Walker (1957) correlated the amino-nitrogen content with the susceptibility of cucumber leaves to angular leaf spot disease caused by *Pseudomonas lachrymans* and concluded that the accumulation of amino nitrogen was mainly related to the day-night temperatures, with the highest concentration occurring at 16 to 28°C (day-night) temperatures. It is therefore conceivable that in the case of the blast disease of paddy also the disease proneness of the host is mainly dependent on its nitrogen metabolism and indeed it must be recalled here that Hashioka (1943*b*, 1944*a, b*) considered temperature as the most important factor in the incidence of the blast disease because of its direct influence on the nitrogen metabolism of the host.

The more rapid enlargement of lesions under greater light intensities lends further support to the importance of the nitrogen metabolism of the host in the biologies of parasitism. Here again the situation has to be largely considered from other works. Greenhill and Chibnall (Chibnall, 1939) found that under well illuminated natural conditions so much glutamine was produced by perennial rye grass, *Lolium perenne*, heavily manured with ammonium sulphate that this amide actually exuded from and crystallized on the surface of the shoots. Yemm (1949) observed that much glutamine and very little asparagine accumulated in illuminated detached barley leaves and he regarded this as further evidence that increased sugar concentration brought about by increased photosynthesis favoured glutamine synthesis. The biochemical mechanism at work here is believed to be the formation of  $\alpha$ -keto-glutaric acid from sugar in the leaves *via* the Kreb's TCA cycle, which combines with ammonia to give glutamine under the agency of glutaminase. From the above considerations it now appears reasonably certain that the rapid enlargement of blast lesions under greater light intensities is evidently due to the formation of glutamine under these conditions. It is further amplified by the fact that the CO 13 paddy plants heavily manured with ammonium sulphate and kept in high light intensities did indeed present an analogous picture like *Lolium perenne* in that glutamine was exuded and crystallized at the tips of the leaves. Moreover the low aspartic acid content observed by Tanaka and Katsuki (1952) at the season of maximum susceptibility of rice seedlings to *Piricularia* is an additional case in point.

That infection failed to take place even in susceptible CO 13 plants grown under ordinary green house conditions even with high doses of ammonium sulphate and the failure of lesions to develop on leaves detached from such plants and floated under light on various sugar solutions, organic acids, nitrogenous salts and ammonium salts of organic acids (conditions that favour glutamine synthesis) can be explainable only on the basis of cell wall resistance under these circumstances. On the basis of such an interpretation of factors which function in the disease proneness of the host, we might expect that with low nyctotemperatures the products of photosynthesis largely accumulate in the leaf itself without any appreciable translocation facilitating the formation of the key amide, glutamine. On the contrary, with high

night temperatures the products of photosynthesis are largely utilized in the formation of more complex polysaccharides of the cell wall like lignin and cutin which make the leaf resistant to mechanical puncture by the germ tube. The inhibitory effect of cuticular excretions on spore germination and the probable microbial antagonism at the leaf surface under these conditions cannot also be ruled out entirely. Under conditions of susceptibility the stimulatory effect of cuticular excretions on spore germination or in so modifying the physico-chemical nature of the leaf surface as to favour appressorial formation are also points to be recognized in evaluating resistance and susceptibility to this disease.

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\*Originals not seen.

# STUDIES ON TRANSPLANTATIONS OF ADULT MOUSE LIVER AND KIDNEY INTO CHICK EMBRYOS

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## ABSTRACT

Grafts of fresh adult mouse liver and kidney were made into chick embryos at the primitive streak stage in the manner described by Waddington (1932).

The grafted embryos were cultured *in vitro* by the technique described by New (1955). The results of the experiments seem to show that fresh adult mouse liver and kidney do not produce inductions into the reacting chick ectoderm.

The influence of kidney grafts on the host tissue has been described here. It appears that the kidney graft upsets the formation of somites in the host.

## INTRODUCTION

Induction by grafts of *Triton* liver, adult mouse kidney and guinea pig kidney in Amphibia has been reported by Chuang (1938) and Toivonen (1940) respectively in connection with regional differentiation of the organiser shown by Spemann (1931). Although much experimental work on chick embryo has been done both in Europe and in America, the progress of Avian Epigenetics has been impeded by technical difficulties. Very recently New (1955) has introduced a simple technique for *in vitro* culture, which has facilitated the study of a variety of grafts on the reacting chick ectoderm.

In the present paper grafts of fresh adult mouse liver and kidney were made into chick embryos.

## MATERIALS AND METHODS

Fertilized fresh hen's eggs obtained from the Government Poultry Farm, Poona, were incubated in an electrically regulated incubator at 37°C to get the primitive streak stage. Necessary precautions were observed by sterilising the glass-ware, instruments etc., and by autoclaving the solutions to avoid contamination.

Fresh liver and kidney of an adult mouse were taken out and washed separately several times in Compton solution to remove blood. A small piece of about 0.3 mm. each, both of liver and kidney, was grafted separately into a chick embryo at the primitive streak stage, in the manner described by Waddington (1932). The embryos were then cultured *in vitro* by the technique described by New (1955). After about 20 hours of culturing, the embryos were fixed in acetic alcohol and serially sectioned at 10 $\mu$ . The sections were stained in Delafield's haematoxylin and differentiated in acid-alcohol.

In all, 20 grafts each of liver and kidney were made and histologically examined.

## DESCRIPTION OF EXPERIMENTS

### *Grafts of Mouse Liver*

In the section shown in Plate XXVII, Fig. 1, the graft (GL) is seen to consist of a mass of hepatic cells. Although the graft is in contact with the host ectoderm, no induction seems to have been caused.

The graft in Plate XXVII, Fig. 2, seems to lie between the endoderm and mesoderm of the host. The graft appears to consist of hepatic cells arranged round the capillaries. As the graft lies between the endoderm and mesoderm, the contact between it and the reacting host ectoderm is prevented and therefore probably no induction is produced.

### *Grafts of Kidney*

The graft in Plate XXVII, Fig. 3, is situated between the ectoderm and mesoderm of the host. The urinary tubules seem to have cut across at several places and are lined by small cubical cells surrounding a small cavity. No induction is caused, although the graft is in contact with the reacting ectoderm of the host. The graft in Plate XXVII, Fig. 4, lies in the coelomic cavity thus preventing the contact between it and the reacting host ectoderm.

In the section shown in Plate XXVII, Fig. 5, the graft lies in between the two mesodermal layers of the host. No induction is produced but the graft seems to have upset the formation of somites. The doubling of somite (S1,S2) is clearly seen on the side occupied by the graft. These somites appear to be bigger than the normal somite seen on the other side. Similar upsetting in the size of the somite was also seen in the sections of some other specimens not shown here. This naturally raises the question as to what is the influence of graft on host tissues which may now be considered.

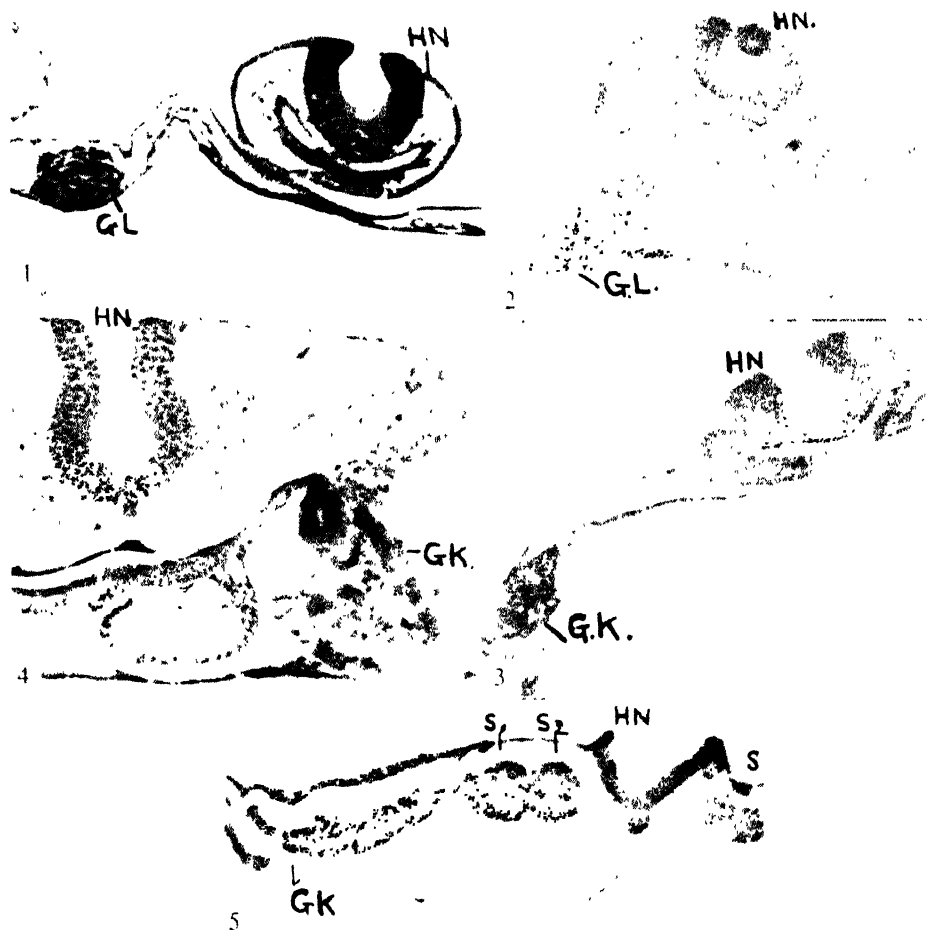
### DISCUSSION

Working with fresh *Triton* liver and mouse kidney in Amphibia, Chuang (1938) found that the *Triton* liver induced trunk structures while the mouse kidney induced cephalic structures. On the contrary, Toivonen (1940) using alcohol treated kidney of the adult guinea-pig, obtained mesodermal inductions. Both believed that different organs are induced by qualitatively different substances. The results of the present work make it probable that the adult fresh mouse liver and kidney do not produce inductions into the reacting chick ectoderm. In several cases (Plate XXVII, Fig. 4) the graft entered the coelomic cavity of the host thus preventing contact between the ectoderm and the graft; but in some other cases (Plate XXVII, Figs. 1, 3), although the graft was in contact with the host ectoderm, induction was not produced. Similarly, in experiments where embryonic *Calotes* liver and kidney were grafted into the chick embryo instead of adult mouse liver and kidney, no induction was caused. It thus appears that the chick ectoderm does not easily react to the evocatory stimulus as does that of Amphibia.

The mutual influence of graft and host on each other is noticeable in Plate XXVII, Fig. 5, where the presence of kidney graft has upset the formation of somites. The doubling of the somite (S1,S2), on the side of which the graft is situated, is clearly seen in Pl. XXVII, Fig. 5. Whether this doubling is due to any specific effect of the graft on the tissues of the host or whether it is merely due to mechanical effect is difficult to state with certainty. However, it may be stated here that such mutual influence of host and graft has been studied in detail by Abercrombie and Waddington (1937), who found a considerable tendency on the part of the graft to become harmoniously incorporated into the host. Waddington (1952) has also recently suggested the possibility of complete incorporation of graft tissue into host's body, causing increase in the size of certain organs.

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Abbreviations. G. L. graft of liver ; G. K. graft of kidney ;  
 H. N., host neural tube ; S, normal somite ;  
 S1, S2 doubling of the somite.

- Fig. 1. Section through the graft and the host neural tube. 130.  
 Fig. 2. Section showing the graft in the coelomic cavity of the host. 130.  
 Fig. 3-4. Section through the graft and the host. 220 and 140 respectively.  
 Fig. 5. Section showing the doubling of the somites. 220.



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# EFFECT OF TRIIODOTHYRONINE ON THE GENITAL ORGANS AND FERTILITY IN MALE RATS\*

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## ABSTRACT

Triiodothyronine (3 and 12  $\mu$ g. daily/18 days) tended to regress the weight of the accessory genital organs in adult male rats without causing any evident alterations in testicular activity. Comparable changes were evoked in the same organs by thyroxine (3 and 12  $\mu$ g. daily/18 days) though in a less conspicuous manner. The nature of action of the two hormones in this respect was similar and the difference between them was only a matter of degree.

Triiodothyronine (12  $\mu$ g. daily/18 days) lowered the weight of the accessory sexual organ of castrated rats and tended to antagonize the typical stimulatory action of testosterone propionate (125  $\mu$ g. daily/12 days) on these organs. The prostatic acid phosphatase activity in the castrated rats was inhibited even further by triiodothyronine. Similarly, the restoration of enzyme activity in the castrates by testosterone propionate was hindered to considerable extent by triiodothyronine.

Triiodothyronine (12  $\mu$ g. daily/18 days) had no effect on fertility in adult male rats.

It was suggested that triiodothyronine (and thyroxine) exerted their effects on accessory male genital organs through an alteration of normal metabolic pattern of androgen. The possibility of a decrease in sensitivity of these organs to androgen under the influence of the thyroid hormones was also considered.

## INTRODUCTION

It has been demonstrated that mild hyperthyroidism induced by optimal physiologic doses of thyroxine (T) results in precocity of the sexual organs in young male rabbits (Maqsood, 1952). Large doses, on the other hand, impede the development of the gonads and accessory sex organs in growing male rabbits and rats (Maqsood, 1952 ; S. N. Roy *et al.*, 1955). Administration of T to mature male rabbits maintained at continuous high temperature proves detrimental to the functioning of the sexual organs (Oloufa *et al.*, 1949). Earlier studies report untoward ponderal changes in the sexual organs of adult male rats after treatment with thyroidal material (Cohen, 1935 ; Smelser, 1939).

It will be evident from the above that while some information is available on the influence of T on the physiology of the male sexual organs, similar knowledge regarding triiodothyronine (TIT) is almost non-existent. This hiatus should be filled in order to gain more insight of the part played by the thyroid in mammalian reproduction. Accordingly, the primary aim of this paper has been to record data on the effect of TIT on the genital organs and fertility in adult male rats. The first part is, however, devoted to a comparative study of the nature of changes elicited in the genital organs by TIT and T. This is followed by an attempt to ascertain whether TIT can exert any influence on the accessory genital organs in the absence of the testes.

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## A. EFFECT OF TIT ON THE GENITAL ORGANS

*Experimental Procedure*

*Animals.* Adult male albino rats of the Institute Colony were used in the experiments reported under this head. The details of grouping of the animals and their body weights are indicated in Tables 1 and 2. The animals were maintained under uniform laboratory conditions throughout the period of investigation.

*TIT and T.* For assessment of their comparative effects, TIT and T were administered in two doses (3 and 12  $\mu\text{g}$ . daily/rat) but the duration of treatment was kept constant for 18 days. However, in the experiment on the possible direct action of TIT on the accessory genital organs only the high dose (12  $\mu\text{g}$ .) was used. The hormones were dissolved in sterile distilled water and were injected by the intramuscular route. The control animals received the solvent alone in a similar manner.

Eartly and Leblond (1954) estimated that the physiological dose of T in adult rats weighing about 150 gm. was between 3 to 6  $\mu\text{g}$ . daily. This finding served as the basis for selection of dosage of T so that the small dose (3  $\mu\text{g}$ .) approximated a physiologic dose. As the initial body weight of the animals was somewhat low (Table 1), the lowest limit of the range provided by these authors (3  $\mu\text{g}$ .) was chosen. The high dose (12  $\mu\text{g}$ .), on the other hand, was meant to be non-physiologic one and as such it was kept as large as twice the upper limit of the range given by Eartly and Leblond. TIT was administered in identical doses (3 and 12  $\mu\text{g}$ .) merely for the sake of comparison, but otherwise the choice of this particular dosage was purely arbitrary.

*Testosterone propionate (TP).* In order to determine whether there was any direct influence of TIT on the accessory genital organs, the rats were castrated and left without any treatment for 10 days. To ensure sufficient regression after the operation these organs were examined by laparotomy before the commencement of treatments. TIT was injected to 2 groups of castrates for 18 days; one of these received *in addition* TP (125  $\mu\text{g}$ . daily/rat in 0.2 c.c. of sterile olive oil) by the intramuscular route for a period of 12 days beginning from the 7th day of TIT administration (Table 2). A third group of castrates was given TP alone at the same dose and by the same route for 12 days. The control animals (normal and castrated) received the solvents alone in a similar manner.

*Biochemical and histological studies.* All the experimental animals were killed 24 hours after the final treatments. The testes, seminal vesicles (SV) and the ventral prostates (VP) were carefully dissected out, weighed to the nearest mg. and finally processed for biochemical and histological studies.

Total cholesterol content of the testis was estimated colorimetrically by a modification (A. C. Roy *et al.*, 1955) of a method by Zlatkis *et al.* (1953). For determination of acid phosphatase activity the VP was homogenized with water (100 ml/gm. fresh tissue), centrifuged for 5 minutes at 2000 r.p.m., and the clear supernatant was finally processed for estimation of enzyme activity by the procedure laid down by Hawk *et al.* (1947).

For histological studies the tissues were fixed in alcoholic Bouin's fluid and serial paraffin sections 6 micra thick, were stained with Ehrlich's hematoxylin followed by eosin.

## RESULTS

*Comparative effects of TIT and T on the genital organs*

*Testis.* It will be evident from Table 1 that both the low and high doses of TIT caused significant reduction in absolute weight of the testis ( $P < 0.05$  and

<0.01 respectively for 3 and 12  $\mu$ g. doses). This was, however, not the case with the relative testis weight. Thus the low dose failed to evoke any significant change, whereas the high dose caused an apparent increase in weight of the organ ( $P < 0.001$ ). Obviously, the latter was related to a sizeable loss in body weight (29.5 gm.) during the experimental period (Table 1) rather than to an actual increase in weight of the organ.

With minor difference in details the *pattern* of influence of T on testis weight was similar to that of TIT. Thus both the doses tended to cause a fall in absolute weight of the organ albeit not in a statistical manner. Similarly, the relative weight registered a significant increase ( $P < 0.001$ ) with the 12  $\mu$ g. dose but the low dose was ineffective in this respect. It was to be noted that the high dose caused a loss of body weight (7.7 gm.) though compared to the corresponding dose of TIT it was negligible (Table 1).

Both TIT and T had no significant effect on total cholesterol concentration of the testis (Table 1).

Histologically, the testis of TIT treated rats was in no way different from that of the controls. The spermatogenesis continued vigorously as in the controls and the Leydig cells did not show any change even after treatment with the 12  $\mu$ g. dose (Pl. XXVIII, Figs. 1 and 2). Similarly, no histological change could be detected in the testis of T treated rats.

*Seminal vesicles.* TIT treatment was accompanied by a significant reduction in absolute and relative weights of the SV ( $P < 0.001$ ). The extent of such reduction was, however, proportional to the dosage : the high dose caused significantly greater loss in weight than the low dose ( $P < 0.001$ ). Similarly, T administration evoked a significant decrease in absolute weight of the organ ( $P < 0.001$ ) and this was dependent on the dosage, the high dose caused significantly greater weight depression than the low dose ( $P < 0.02$ ). However, the relative weight did not follow quite the same pattern as the low dose of T significantly reduced the SV weight ( $P < 0.05$ ) but the high dose though tended to do the same, yet the difference from the controls in this respect was statistically insignificant. Further, the low dose caused an apparently greater decrease in relative weight of the organ but again, the difference was not statistically significant (Table 1). It was interesting that TIT and T did not differ significantly in their capacity to depress the SV weight (absolute and relative) when injected at 3  $\mu$ g. dose ; but the high dose (12  $\mu$ g.) of TIT caused significantly greater reduction in weight of the organ (absolute and relative) than an equivalent dose of T ( $P < 0.001$ ). On percentage basis too, 12  $\mu$ g. dose of TIT caused as much as 80.7 per cent reduction in absolute SV weight in contrast to a mere 39.7 per cent depression after administration of the same dose of T. The corresponding figures for changes in relative weight of the organ were 69.3 and 20.1 per cent reductions after treatment with TIT and T (12  $\mu$ g.) respectively. Similar figures for the low dose (3  $\mu$ g.) were inconspicuous and did not reveal much difference between the two hormones with respect to their capacity to influence relative SV weight.

Histologically, the SV of rats injected with 3  $\mu$ g. dose of TIT were similar to those of the controls. In animals treated with the high dose, however, the villi-like folds of this organ appeared somewhat stunted and the glandular epithelium showed signs of atrophy at certain places resembling that of the castrates (Pl. XXVIII, Figs. 3 and 4). Nevertheless, such atrophy of the epithelium was focal and not generalized as in the castrates (Pl. XXVIII, Fig. 5). In general, the organ appeared normal and active ; the secretion granules were abundant in the epithelium and the lumen was gorged with copious amounts of secretions as in the controls. The SV of T treated rats were indistinguishable from those of the controls.

*Ventral prostate.* TIT administration at the two doses caused a significant depression of VP weight, both absolute and relative ( $P < 0.001$ ). As with the SV weight, the extent of this depression was dependent on the dosage ; the high

dose evoked a significantly greater reduction than the low dose ( $P < 0.001$ ). T also inhibited VP weight, both absolute and relative ( $P < 0.001$ ); and the degree of reduction in absolute weight at least, tended to be proportional to the dosage (Table 1). The high dose apparently caused greater reduction than the low dose though the difference was not statistically significant (Table 1). The extent of loss of relative weight of the organ under the influence of T (3 and 12  $\mu$ g. doses) was, however, of the same order. A dose for dose comparison of TIT and T revealed that they did not differ in their ability to inhibit VP weight when administered at 3  $\mu$ g. dose. The high dose of TIT, on the other hand, evoked significantly greater loss in absolute weight than the corresponding high dose of T. The relative weight of the organ responded more or less similarly to the high dose of the two hormones and the difference in weight was statistically insignificant (Table 1). Here was therefore a little quantitative deviation from the pattern of ponderal changes elicited in the SV by the two thyroid hormones. On percentage basis, 12  $\mu$ g. TIT caused 66 per cent reduction in absolute weight of the VP in contrast to 49.3 per cent decrease after treatment with an equivalent dose of T. Similar figures for the relative weight of the organ were 47.8 and 34.6 per cent depressions after administration of TIT and T respectively.

Prostatic acid phosphatase activity did not show any significant change after injection of the low dose of TIT (Table 1); the high dose, on the other hand, caused a significant rise in enzyme activity ( $P < 0.02$ ). In contrast, T had virtually no influence on acid phosphatase activity in the VP.

The low dose of TIT did not evoke any histological change in the VP. The high dose, however, caused atrophy of the glandular epithelium in some aeni (Pl. XXVIII, Figs. 6 and 8); in others the epithelium was normal as in the controls. There was no generalized shrinkage of the aeni and regression of the glandular epithelium, so commonly encountered in the VP of castrated rats (Pl. XXVIII, Fig. 7). No histologic change could be detected in the VP of rats treated with T.

#### *Effect of TIT on the seminal vesicles and the ventral prostate of castrated rats*

*Seminal vesicles.* It will be evident from Table 2 that castration caused the expected regression in SV weight, both absolute and relative ( $P < 0.001$ ). TP therapy to castrates increased the weight of the organ even beyond the normal range so that the difference from the controls was statistically significant ( $P < 0.001$ ). TIT administration to castrated rats caused even further decrease in absolute SV weight and in this respect the difference from the castrates was statistically significant ( $P < 0.001$ ). However, the relative weight of the organ in the castrates though tended to fall even further after TIT treatment, yet was not low enough to be significantly different from the untreated castrates. It was worth noting that there was a marked loss of body weight (31.7 gm.) in these animals (Table 2). When TP was administered to castrated rats in addition to TIT, the characteristic increase in absolute SV weight was considerably inhibited. So much so, that the group treated with TIT and TP conjointly had a significantly lower SV weight (absolute) than that of the animals injected TP alone ( $P < 0.001$ ). This was not the case with the relative weight of the organ which though tended to be lower in the conjoint group, yet was not significantly different from the animals which received TP alone (Table 2). It was of interest that the body weight of the animals injected with the two hormones simultaneously showed a loss during the treatment period (28.4 gm.).

Castration caused the expected regression of histological structure of the seminal vesicles (Pl. XXVIII, Figs. 3 and 5) but TP therapy was accompanied by a return of the organ to normalcy. The histologic picture of the castrates treated with TIT was similar to that of the untreated castrates. Likewise, no difference in the histologic structure of the seminal vesicles could be detected in the group given TP alone or TP in conjunction with TIT.

*Ventral prostate.* Castration caused the characteristic depression of VP weight, both absolute and relative ( $P < 0.001$ ). TP therapy stimulated the organ considerably so that its weight (absolute and relative) exceeded that of the controls ( $P < 0.001$ ). Unlike the seminal vesicles, the administration of TIT to castrated rats had no effect on the absolute weight of the VP (Table 2). When, however, TP was added to TIT the absolute prostatic weight was stimulated to a significantly lesser degree than when TP was given alone ( $P < 0.001$ ). The relative weight of the organ responded somewhat differently to TIT treatment. Thus the injection of TIT alone to castrates apparently elevated the weight of the organ and the difference from the untreated castrates was statistically significant ( $P < 0.001$ ). But in the group which received TIT and TP conjointly, the stimulation of relative VP weight tended to be less than in the group treated with TP alone, though the difference was not statistically significant (Table 2). It should, however, be recalled that TIT caused a loss of body weight whether given alone or in combination with TP.

Acid phosphatase activity in the VP declined significantly after castration ( $P < 0.001$ ) but TP therapy restored the enzyme activity to normal level. Administration of TIT to castrates caused a further fall in phosphatase activity so that the difference from the untreated castrates was statistically significant ( $P < 0.02$ ). When TP was given along with TIT the elevation of enzyme activity was somewhat less than in the castrates treated with TP alone; but the difference was not statistically significant. On percentage basis, castration caused 41.8 per cent inhibition of phosphatase activity from the controls; whereas TIT therapy to castrates evoked as much as 72.1 per cent reduction. The enzyme activity in the castrates treated with TIT was 52 per cent less than in the untreated castrates. It was of added interest that phosphatase activity in the VP of castrates injected with TP was 67.7 per cent higher than in the untreated castrates whereas, in the group treated with TIT along with TP the enzyme activity was only 21.7 per cent higher.

Histologically, castration caused the characteristic regressive changes in the VP (Pl. XXVIII, Figs. 6 and 7) but TP therapy restored the histological picture of the organ to normalcy. The histological structure of the VP of animals injected with TIT was similar to that of the untreated castrates. Likewise, the histological appearance of the organ in the group treated with TIT and TP conjointly was indistinguishable from that of the animals injected with TP alone.

## B. EFFECT OF TIT ON FERTILITY

### *Experimental Procedure*

*Animals.* Adult male and female albino rats of the Institute Colony were used in this study (Table 3). The animals were of proved fertility. They were maintained under uniform laboratory conditions throughout the experimental period.

*TIT.* The hormone was injected (to male rats) by the intramuscular route in a dosage of 12  $\mu$ g. daily per rat (in sterile distilled water) for 18 days. Out of this total period of treatment, the first 10 daily injections were given *before mating* and the remaining 8 during the stage when the animals were actually caged with the females. The control males and the females received the solvent alone in a similar manner.

*Examination of vaginal smears.* Before the animals were put to mating the vaginal smears of all the females were examined daily for 3 complete estrus in order to ensure its cyclic recurrence. A female showing any irregularity of the cycle was discarded. The average length of the estrus cycle was 4.7 days. The subsequent daily examination of vaginal smears started from the very first day of cohabitation and continued till pregnancy was detected.

*Mating.* For mating, 2 females were caged with a male according to the method of Kai (1957). The day on which a vaginal smear contained spermatozoa, was considered as the day of mating. About 13 to 16 days after cohabitation and every 2 to 3 days thereafter, the females were palpated and weighed for detection of pregnancy. No limited mating test period was employed.

*Examination of spermatozoa and histological studies.* The males were caged with the females for 8 days, that is, for the latter part for the hormone treatment period. They were removed on the 9th day (that is one day following the cessation of TIT injections) and killed by decapitation. The spermatozoa of each control and treated males were collected from the middle segment of the vas deferens and were examined for motility and any gross abnormality. The testes, SV, and VP were dissected out, weighed to the nearest mg. and finally fixed for histological studies. The procedure employed for the latter was the same as before.

## RESULTS

Table 3 shows that TIT treatment had no effect on fertility of male rats. All the treated males mated successfully and there was no delay in mating. The latter was evident from the fact that those injected with TIT mated on an average of 3.0 days from cohabitation but the corresponding figure for the controls was virtually the same (3.3 days). The number of young born to two groups of females was the same whether mated with the control or TIT treated males.

The motility of the spermatozoa appeared normal in animals injected with TIT; and although no quantitative estimation was made yet, it was possible to judge from gross microscopical examination that the number of abnormal sperms was equally low in the two groups. There was no preponderance of any particular type of abnormal sperms in the TIT treated animals.

TIT administration caused a significant decrease only in the absolute weight of the testis ( $P < 0.05$ ) but not in its relative weight. Histological picture of the testis was, however, normal. There was a loss of body weight (66.3 gm.) in the hormone treated animals (Table 3).

SV weight (both absolute and relative) registered a significant fall after TIT administration ( $P < 0.001$ ). On percentage basis, TIT injection caused 54 per cent reduction in absolute weight but the corresponding figure for the relative weight was 43.8 per cent. Histological changes similar to those reported previously (see Pl. XXVIII, Fig. 4) were seen in the SV of TIT treated animals.

The absolute and relative ventral prostate weight declined significantly after TIT injections ( $P < 0.001$ ). The extent of this reduction was 57.1 per cent for the absolute weight and 48.4 per cent for the relative weight. The histological picture of the VP of TIT treated animals was comparable to that of the previous animals injected with the same hormone.

## DISCUSSION

The data presented in this report indicated that TIT had no appreciable effects on the testis of adult male rats irrespective of the dosage in which it was administered. No histological changes were seen in the gametogenic or endocrine parts of the organ. Spermatogenesis continued vigorously as in the controls and the interstitial elements did not show any abnormality. Whatever ponderal changes occurred in the testis could largely be ascribed to the influence of TIT on body weight rather than to a true effect on the organ itself. Added to these, the cholesterol concentration of the testis remained unaltered after hormone treatment which suggested that the endocrine status of the organ did not undergo any fundamental change. The recognized rôle of cholesterol in the biogenesis of

androgens (Dorfman and Shipley, 1956) did not seem to carry such a view beyond the realm of facts.

It was significant that T exerted virtually similar effects on the testis when injected in identical doses. Untoward histological or metabolic changes indicative of a deranged functional status of the organ were absent. In this connection S.N. Roy *et al.* (1955) reported that T (12.5  $\mu$ g. daily) provoked testicular atrophy in early puberal rats. Obviously, the age factor was responsible for the different nature of response of the testis to the same hormone (12  $\mu$ g. daily) observed in the present study.

In contrast to the testis, the accessory genital organs showed some noteworthy changes after TIT treatment. Thus the weight of the SV was reduced drastically with the high dose and focal atrophic changes of the castrate type were encountered consistently in the glandular epithelium. Nevertheless, such changes were not generalized as in the castrates and the net histologic picture was one of an organ with uninterrupted secretory activity. Similar ponderal changes with focal regression of the acinar epithelium were seen in the VP but again, the overall secretory activity appeared to be normal. Further, acid phosphatase activity in the gland did not show any significant change after administration of low dose of TIT but curiously, the high dose caused a significant rise in enzyme activity. The prostatic acid phosphatase was believed to bear a positive correlation with androgenicity (McCullagh and Schaffenburg, 1954) and on this basis the acceleration of enzyme activity after TIT administration could be regarded as an indication of enhanced androgen output by the testis. This was, however, unlikely as the VP weight diminished rather than increased in response to hormone treatment. This anomalous rise in acid phosphatase activity was therefore due to some unknown cause.

A comparison of the above with the effects produced by T on accessory genital organs led to interesting findings. Firstly, the absolute SV weight also tended to decline, but not to the same extent as in the TIT treated animals. The relative weight of the organ too showed some inhibition but again, in a less prominent manner than after TIT administration. However, unlike the latter T caused no appreciable histologic change in the organ. Similarly, the VP weight diminished after T injection and the difference with TIT, if any, was only a matter of degree. However, no histologic changes were encountered in this organ nor did acid phosphatase activity undergo any aberrations. Nevertheless, such comparative details brought into relief two moot points regarding the nature of action of these hormones on the genital organs. These were: (1) both the hormones evoked inhibitory changes in the weight of the accessories particularly at the high dose, without the mediation of the testis, and (2) such changes were unaccompanied by any commensurate lowering of the functional potentiality of these organs. The evidence bearing upon these points will be considered in the subsequent paragraphs.

In the first place, it was noticed that TIT (at the high dose) tended to lower the SV weight of castrated rats: the absolute weight registered a marked fall but the relative weight showed only a modicum of reduction. However, the histological picture of the organ was indistinguishable from that of the untreated castrates and the body weight of the treated animals showed a loss during the experimental period. These facts suggested that the ponderal changes observed in the SV of castrated rats were due to a general catabolic effect of TIT on the body weight rather than to any direct influence on the organ *per se*. The lack of response of the absolute VP weight or an apparent increase in relative weight of this organ (in the castrates treated with TIT), tended to eliminate any possible direct inhibitory action of TIT. Similarity in histological picture of the VP in the castrates either untreated or injected with TIT, was also to be considered.

Nevertheless, it was significant that prostatic acid phosphatase acidity declined to the extent of 52 per cent when the TIT was given to the castrates. This was indeed a powerful evidence in favour of a direct action of this hormone on the VP.

The results of TP therapy revealed certain facts which had important bearing on this issue. The androgen caused characteristic stimulation of the accessories in the castrates, but when TIT was given along with it the degree of such stimulation was less. It was of added interest that prostatic acid phosphatase activity in the former group (TP alone) was 67.7 per cent higher than in the untreated castrates, whereas the corresponding figure for the latter group (TP in combination with TIT) was as low as 21.7 per cent. However, the loss of body weight in the combined treated animals was not to be ignored.

A careful consideration of the evidence *pro and con*, led to the conclusion that TIT was capable of exerting some direct influence on accessory sexual organs of adult male rats. Further, the results of experiments with castrates suggested an antagonistic action of TIT on TP at the periphery. The recent findings of Bradlow *et al.* (1956) on the influence of TIT on the metabolism of androgens seemed pertinent in this connection. These authors noted that TIT favoured an increased excretion of androsterone whether from endogenous sources or from androgens introduced into the body exogenously. This increase in urinary excretion was believed to be due to an enhanced rate of formation of this androgen metabolite. Conversely, hypothyroidism was shown to elevate the rate of urinary excretion of etiocholanolone. The inhibitory action of TIT (and T) on the accessory genital organs of intact rats was probably due to a similar alteration in metabolic pattern of testicular androgen. A similar explanation could be offered for the failure of TP to exert its maximal effects on the accessory genital organs of castrates in the presence of TIT. Alternatively, it could be postulated that the reactivity of these organs to androgen declined after TIT treatment. Smelser's (1939) findings on the increased androgen requirement of castrated rats injected with T tacitly conveyed a similar idea. Moreover, Ford *et al.* (1957) failed to locate autoradiographically  $^{131}$ I-labelled TIT (and T) in the interstitium of the guineapig testis although considerable accumulation of these hormones occurred in the prostatic epithelium. It was, however, possible that a casual relationship existed between the two alternatives.

TIT treatment did not interfere with fertility even though the accessory sexual organs of the recipients showed considerable regression. The motility of the spermatozoa was unaffected and there was no preponderance of any particular type of abnormality. It was interesting that T did not interfere with the  $O_2$  consumption of spermatozoa in several mammalian species (Maqsood, 1952). These facts, therefore, indicated that whatever might have been the nature and extent of direct effects of TIT it did not compromise the functional potentiality of the accessory sexual organs.

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TABLE

*The weight of the genital organs, cholesterol content of the testis and acid phosphatase*

| Treatment               | Mean testis weight with S.E. |                                    | Mean seminal vesicle weight with S. E. |                                    |
|-------------------------|------------------------------|------------------------------------|--|------------------------------------|
|                         | Absolute (Mg.)               | Relative (Mg./100 gm. body weight) | Absolute (Mg.)                         | Relative (Mg./100 gm. body weight) |
| Controls (solvent only) | 1160.0 $\pm$ 41.48<br>(10)*  | 719.4 $\pm$ 19.89<br>(10)          | 247.1 $\pm$ 19.77<br>(10)              | 163.5 $\pm$ 14.60<br>(10)          |
| TIT (3 $\mu$ g. daily)  | 1030.0 $\pm$ 19.64<br>(10)   | 724.7 $\pm$ 24.05<br>(10)          | 163.2 $\pm$ 10.12<br>(10)              | 114.9 $\pm$ 8.11<br>(10)           |
| TIT (12 $\mu$ g. daily) | 937.0 $\pm$ 26.34<br>(10)    | 940.4 $\pm$ 51.58<br>(10)          | 47.7 $\pm$ 5.97<br>(10)                | 50.1 $\pm$ 6.19<br>(10)            |
| T (3 $\mu$ g. daily)    | 1083.0 $\pm$ 33.84<br>(10)   | 693.3 $\pm$ 11.50<br>(10)          | 175.0 $\pm$ 6.11<br>(10)               | 112.8 $\pm$ 5.47<br>(10)           |
| T (12 $\mu$ g. daily)   | 995.8 $\pm$ 35.32<br>(10)    | 887.2 $\pm$ 28.97<br>(10)          | 148.8 $\pm$ 7.29<br>(10)               | 130.6 $\pm$ 7.59<br>(10)           |

\* Figure in parenthesis indicates the number of animals.

TABLE

*Seminal vesicle and ventral prostate weights, and acid phosphatase activity in the*

| Treatment  | Mean seminal vesicle weight |                                    | Mean ventral prostate weight |                                    |
|--|-----------------------------|------------------------------------|------------------------------|------------------------------------|
|  | Absolute (Mg.)              | Relative (Mg./100 gm. body weight) | Absolute (Mg.)               | Relative (Mg./100 gm. body weight) |
| Controls (solvent only)                                      | 153.7 $\pm$ 5.29**<br>(6)*  | 98.8 $\pm$ 4.59<br>(6)             | 171.3 $\pm$ 5.41<br>(6)      | 109.4 $\pm$ 2.95<br>(6)            |
| Castrate (solvent only)                                      | 60.1 $\pm$ 7.25<br>(7)      | 34.4 $\pm$ 4.34<br>(7)             | 25.5 $\pm$ 1.43<br>(7)       | 14.6 $\pm$ 1.10<br>(7)             |
| Castrate + TP (125 $\mu$ g. daily)                           | 323.9 $\pm$ 13.84<br>(6)    | 185.2 $\pm$ 12.57<br>(6)           | 332.3 $\pm$ 10.19<br>(6)     | 189.3 $\pm$ 9.05<br>(6)            |
| Castrate + TIT (12 $\mu$ g. daily)                           | 36.9 $\pm$ 1.84<br>(7)      | 29.8 $\pm$ 1.41<br>(7)             | 25.05 $\pm$ 0.63<br>(7)      | 20.2 $\pm$ 0.61<br>(7)             |
| Castrate + TIT (12 $\mu$ g. daily) + TP (125 $\mu$ g. daily) | 221.7 $\pm$ 17.17<br>(8)    | 161.9 $\pm$ 14.11<br>(8)           | 203.7 $\pm$ 10.35<br>(7)     | 169.9 $\pm$ 9.52<br>(7)            |

\* Figure in parenthesis indicates the number of animals. \*\* S.E. of the mean.

TABLE

*Effect of TIT on the weight of the genital organs and*

| Treatment               | No. of males fertile | No. of females conceived | Days between cohabitation and mating.<br>Mean (range) | Mean No. of young per litter | Mean testis weight with S.E. |                                    |
|-------------------------|----------------------|--------------------------|---|------------------------------|------------------------------|------------------------------------|
|                         |                      |                          |   |                              | Absolute (Mg.)               | Relative (Mg./100 gm. body weight) |
| Controls (solvent only) | 6                    | 12                       | 3.3 (2-5)   | 8.7                          | 1162.8 $\pm$ 35.63<br>(6)*   | 604.7 $\pm$ 25.31<br>(6)           |
| TIT (12 $\mu$ g. daily) | 8                    | 15                       | 3.0 (1-5)   | 8.3                          | 1034.1 $\pm$ 40.21<br>(8)    | 653.9 $\pm$ 14.33<br>(8)           |

\* Figure in parenthesis indicates the number of animals.

1

*activity in the ventral prostate of TIT and T treated rats*

| Mean ventral prostate weight<br>with S. E. |  | Mean testis<br>cholesterol con-<br>tent with S.E.<br>(Mg./gm. testis) | Acid phosphatase<br>activity of<br>ventral prostate<br>with S.E.<br>(Mg. P/Gm/<br>1 hour). | Mean body weight (Gm.)<br>with S.E. |                    |
|--|--|---|--|-------------------------------------|--------------------|
| Absolute<br>(Mg.)                          | Relative<br>(Mg./100 gm.<br>body weight) |   |  | Initial                             | Final              |
| 260.4±14.78<br>(10)                        | 176.7±13.54<br>(10)                      | 5.01±0.10<br>(7)  | 1.15±0.20<br>(6)   | 123.8±3.38<br>(10)                  | 152.8±3.83<br>(10) |
| 165.5±14.68<br>(10)                        | 115.8±9.95<br>(10)                       | 4.82±0.14<br>(7)  | 1.47±0.06<br>(6)   | 122.5±3.43<br>(10)                  | 143.7±4.73<br>(10) |
| 88.5±6.85<br>(10)                          | 92.2±6.95<br>(10)                        | 5.10±0.14<br>(7)  | 1.86±0.15<br>(7)   | 125.5±3.76<br>(10)                  | 96.0±2.74<br>(10)  |
| 170.8±16.65<br>(10)                        | 108.7±9.40<br>(10)                       | 5.02±0.19<br>(6)  | 1.11±0.07<br>(7)   | 130.5±3.12<br>(10)                  | 157.2±4.88<br>(10) |
| 131.8±9.98<br>(10)                         | 115.5±8.93<br>(10)                       | 4.75±0.11<br>(7)  | 1.24±0.07<br>(7)   | 122.3±2.74<br>(10)                  | 114.6±2.38<br>(10) |

2

*ventral prostate of castrated rats treated with TIT*

| Acid phosphatase<br>activity of the<br>ventral prostate<br>(Mg. P/gm./1 hour) | Mean body weight (gm.) |                   |
|---|------------------------|-------------------|
|   | Initial                | Final             |
| 1.65±0.24<br>(6)  | 155.0±3.73<br>(6)      | 156.1±3.92<br>(6) |
| 0.96±0.08<br>(6)  | 154.0±4.20<br>(7)      | 176.8±7.2<br>(7)  |
| 1.61±0.11<br>(6)  | 152.1±4.86<br>(6)      | 176.5±5.84<br>(6) |
| 0.46±0.14<br>(6)  | 155.7±4.13<br>(7)      | 124.0±4.61<br>(7) |
| 1.26±0.20<br>(6)  | 166.6±4.39<br>(8)      | 138.2±5.4<br>(8)  |

3

*fertility in male rats*

| Mean seminal vesicle weight<br>with S.E. |  | Mean ventral prostate weight<br>with S.E. |  | Mean body weight (Gm.)<br>with S.E. |                   |
|--|--|---|--|-------------------------------------|-------------------|
| Absolute<br>(Mg.)                        | Relative<br>(Mg./100 gm.<br>body weight) | Absolute<br>(Mg.)                         | Relative<br>(Mg./100 gm.<br>body weight) | Initial                             | Final             |
| 224.6±10.37<br>(6)                       | 116.4±4.71<br>(6)                        | 388.4±10.67<br>(6)                        | 202.8±11.78<br>(6)                       | 199.5±7.08<br>(6)                   | 193.5±8.11<br>(6) |
| 103.5±7.03<br>(8)                        | 65.4±3.85<br>(8)                         | 166.3±10.96<br>(8)                        | 104.6±4.98<br>(8)                        | 224.5±6.68<br>(8)                   | 158.2±5.55<br>(8) |

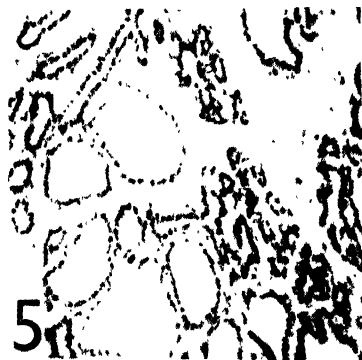
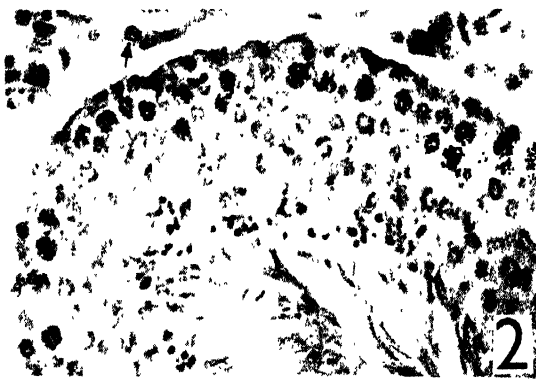
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## EXPLANATION OF PLATE XXVIII

(All figures are photomicrographs. Figs. 1-2 are magnified  $\times 350$  ; figs. 3-8 are magnified  $\times 170$ )

- Fig. 1.—Testis of a control rat. Note full spermatogenesis. Leydig cells are indicated by arrow.
- Fig. 2.—Testis of a TIT treated rat (12  $\mu$ g. daily). Full spermatogenesis. Leydig cells (indicated by arrow) are normal.
- Fig. 3.—Seminal vesicles of a control rat. Note prominent villi-like folds lined by secretory epithelium. The lumen is full of secretions (right lower end of the fig.).
- Fig. 4.—Seminal vesicles of a TIT treated rat (12  $\mu$ g. daily). Note somewhat stunted villi and focal atrophy of the secretory epithelium (indicated by arrow on left-hand side of the fig.). However, the epithelium is mostly normal and active at other places (indicated by arrow on the right-hand side of the fig.). Abundant secretion in the lumen.
- Fig. 5.—Seminal vesicles of a castrated rat. Note the generalized atrophy of the epithelium. Compare with Fig. 4.
- Fig. 6.—Ventral prostate of a control rat. Note large acini lined by secretory epithelium. The lumen of the acini is full of secretions.
- Fig. 7.—Ventral prostate of a castrated rat. Note shrinkage of the acini and atrophy of the secretory epithelium. The lumen is full of coagulated secretions.
- Fig. 8.—Ventral prostate of a TIT treated rat (12  $\mu$ g. daily). Note atrophy of the secretory epithelium in some acini (indicated by arrow at the left-hand side of the fig.) ; other acini show normal epithelium (indicated by arrow at the right-hand side of the fig.) Compare with figs. 6 and 7.





A CONTRIBUTION TO THE KNOWLEDGE OF THE DIATOMACEAE  
OF KANYA KUMARI (CAPE COMORIN), INDIA—II

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(Communicated by M. S. Randhawa, F.N.I.)

(Received May 14; read August 30, 1958)

In his previous paper (Venkataraman, 1957), the author described 29 forms of Diatoms mainly belonging to the order Pennales from Kanya Kumari (Cape Comorin). The present communication deals with the description of 28 additional forms representing 12 genera, mainly belonging to the order Centrales, from the same locality.

SYSTEMATIC ENUMERATION

1. *Stephanopyxis palmeriana* (Greville) Grunov. Hustedt, 1930, p. 308, fig. 147; Subrahmanyam, 1946, p. 88, figs. 12-14, 17, 18, 20.

Frustules linked in chains; cells cylindrical; valves slightly convex; cells joined together by their spines; spines many (Fig. 1).

Diam. frustule, 72.2-76  $\mu$ .

Areolae at the base, 6-7 in 10  $\mu$ .

Areolae at the disc, 4-5 in 10  $\mu$ .

Habitat: Planktonic. June 15, 1956.

2. *Skeletonema costatum* (Greville) Cleve. Van Héurck, 1889, p. 437, Pl. XXXIII, fig. 889, 890; Hustedt, 1930, p. 311, fig. 149; Subrahmanyam, 1946, p. 89, figs. 7, 8, 10; Venkataraman, 1939, p. 297, fig. 6.

Frustules linked in chains by means of marginal spines; cells lens-shaped with rounded ends, weakly silicified (Fig. 2).

Diam. frustule, 7.6-10.5  $\mu$ .

Habitat: Planktonic. June 15, 1956.

3. *Hyalodiscus scoticus* (Kütz) Grunov. Hustedt, 1930, p. 293, fig. 131; Misra, 1956, p. 538, fig. 6.

Frustules linked in chains; cells lens shaped; membrane finely striated; striations punctate (Fig. 3).

Diam. frustule, 28.5-34.2  $\mu$ .

Striae, 12-15 in 10  $\mu$ .

Habitat: Epiphytic on *Chaetomorpha* sp. June 17, 1956.

This form agrees well with the Dwaraka form except in the bigger striations and punctae.

4. *Thalassiosira decipiens* (Grun) Jörgenson. Hustedt, 1930, p. 322, fig. 158; Subrahmanyam, 1946, p. 89, fig. 19.

Valves flat, areolated; cells disc-shaped (Fig. 6).

Diam. frustule, 15-16.5  $\mu$ .

Areolae, 12-15 in 10  $\mu$ .

Habitat: Planktonic. June 15, 1956.

5. *Cyclotella striata* (Kütz) Grunov. Van Héurck, 1899, p. 444, Pl. XXII, fig. 651; Hustedt, 1930, p. 344, fig. 176; Subrahmanyam, 1946, p. 92, fig. 31.

Valves discoid and evenly striated ; coarsely punctate in the centre (Fig. 4).

Diam. frustule, 19–38  $\mu$ .

Striae, 8–12 in 10  $\mu$ .

Habitat : Planktonic. June 15, 1956.

6. *Cyclotella meneghiniana* Kütz. Van Héurck, 1899, p. 447, p. XXII, fig. 656 ; Hustedt, 1939, p. 341, fig. 174 ; Subrahmanyam, 1946, p. 92, fig. 25, 26, 27 ; Venkataraman, 1939, p. 299, fig. 11, 14 ; Misra, 1956, p. 540, fig. 9.

Valves disc-shaped ; margin radially ribbed ; striation coarse and wedge-shaped (Fig. 5).

Diam. frustule, 22.8–24.9  $\mu$ .

Striae, 9 in 10  $\mu$ .

Habitat : Planktonic. June 15, 1956.

7. *Coscinodiscus finicus* Misra. Misra, 1956, p. 541, fig. 10. Frustules discoid with polygonal areolation ; areoles arranged in tangential rows as in the type ; margin strongly striated (Fig. 7).

Diam. frustule, 38–39.9  $\mu$ .

Striae, 9 in 10  $\mu$ .

Habitat : On bottom mud of saline pools. June 18, 1956.

8. *Coscinodiscus granii* Cough. Hustedt, 1930, p. 436, fig. 237 ; Venkataraman, 1939, p. 300, Pl. XVII ; figs. 2, 16, 17 ; Subrahmanyam, 1946, p. 96, figs. 33, 35, 39.

Valves rounded, areolated ; in the girdle view wedge-shaped ; areolations bigger in the centre forming a rosette (Fig. 8).

Diam. frustule, 165  $\mu$ .

Areolae, 5–6 in 15  $\mu$ .

Habitat : On bottom mud of saline pools. June 18, 1956.

9. *Coscinodiscus sublineatus* Grunov. Hustedt, 1930, p. 394, fig. 205 ; Subrahmanyam, 1946, p. 95, fig. 34.

Valves rounded with hexagonal areolae (Fig. 9).

Diam. frustule, 22.5  $\mu$ .

Habitat : On bottom mud of saline pools. June 18, 1956.

10. *Coscinodiscus lineatus* Ehrén. Hustedt, 1930, p. 392, fig. 204 ; Subrahmanyam, 1946, p. 94, figs. 24, 28.

Valves slightly convex or concave, areolated ; margin of the valve striated (Fig. 10).

Diam. frustule, 52.5  $\mu$ .

Areolae, 5–10 in 10  $\mu$ .

Habitat : On bottom mud of saline pools. June 18, 1956.

11. *Coscinodiscus asteromphalus* Ehrén. Hustedt, 1930, p. 452, fig. 250 Van Héurck, 1899, p. 530, fig. 277 ; Subrahmanyam, 1946, p. 99, figs. 62–65.

Valves disc-shaped, areolated with a clear area in the centre ; outer membrane punctate ; margin striated (Fig. 11).

Diam. frustule, 399  $\mu$ .

Areolae, 4–5 in 10  $\mu$ .

This form has very big frustules and slightly greater number of areolae than in the type.

Habitat : On bottom mud of saline pools. June 18, 1956.

12. *Rhizosolenia crassispina* Schroeder. Subrahmanyam, 1946, p. 119, figs. 138, 139.

Cells cylindrical ; valves tapering ; spines slightly constricted at the base ; chloroplasts numerous (Fig. 15).

Diam. frustule, 33–45.6  $\mu$ .

Habitat : Planktonic. June 15, 1956.

13. *Rhizosolenia cylindrus* Cleve. Hustedt, 1930, p. 572, fig. 325; Subrahmanyam, 1946, p. 114, figs. 111-112.

Cells cylindrical; valves conical; setae bent (Fig. 14).

Diam. frustule, 24.7  $\mu$ .

Habitat: Planktonic. June 15, 1956.

14. *Rhizosolenia stouterforthii* Peragallo. Van Héurek, 1899, p. 416; Hustedt 1930, p. 578, fig. 329; Subrahmanyam, 1946, p. 115, figs. 113, 115, 117.

Cells cylindrical; prevalvar axis bent; valves with small spines; intercalary bands many (Fig. 13).

Diam. frustule, 15-18  $\mu$ .

Length frustule, 135  $\mu$ .

Habitat: Planktonic. June 15, 1956.

This form has much narrower cells than the type.

15. *Bacteriastrum cosmosum* Pavillard. Hustedt, 1930, p. 622, fig. 361; Subrahmanyam, 1946, p. 126, figs. 178-78.

Cells cylindrical; inner setae bent towards the posterior end and run parallel to the chain axis; anterior terminal setae with spirally arranged spines, curved towards the posterior; setae 8-12 (Fig. 12).

Diam. frustule, 9.5-15.2  $\mu$ .

Habitat: Planktonic. June 15, 1956.

This form has slightly narrower frustules.

16. *Bacteriastrum hyalinum* Lauder. Hustedt, 1930, p. 615, fig. 354; Subrahmanyam, 1946, p. 125-6, figs. 164, 166, 167, 169, 173.

Cells short; setae many; terminal setae bent over the chain axis (Fig. 16).

Diam. frustule, 34.2-38  $\mu$ .

Habitat: Planktonic. June 15, 1956.

17. *Bacteriastrum hyalinum* var. *princeps* (Cast) Ikari. Hustedt, 1930, p. 615, fig. 355; Subrahmanyam, 1946, p. 127, figs. 165, 168.

Cells short; setae spirally twisted (Fig. 17).

Diam. frustule, 22.8-24.7  $\mu$ .

Habitat: Planktonic. June 15, 1956.

18. *Chaetoceros affinis* var. *intermedius* Subrahmanyam. Subrahmanyam, 1946, p. 137, fig. 233.

Cells linked in chains; setae hair like and slightly bent towards the distal end; inner and end setae similar (Fig. 18).

Length apical axis, 7.6-11.4  $\mu$ .

Habitat: Planktonic. June 15, 1956.

19. *Chaetoceros lorenzianus* Grunov. Hustedt, 1930, p. 679, fig. 385; Subrahmanyam, 1946, p. 131, figs. 198, 199, 202-4, 206-9.

Cells linked in chains; setae arise from the corners; terminal setae thicker than the inner ones; setae punctate areolate (Fig. 21).

Length apical axis, 67.6  $\mu$ .

Habitat: Planktonic, June 15, 1956.

20. *Chaetoceros lacinosus* Schütt. Hustedt, 1930, p. 701, fig. 401a; Subrahmanyam, 1946, p. 139, figs. 228, 229, 231.

Cells linked in chains; apertures oblong; terminal setae thicker and parallel to the chain axis (Fig. 20).

Length apical axis, 10.5-22.6  $\mu$ .

Habitat: Planktonic. June 15, 1956.

21. *Chaetoceros didymus* Ehrén. Hustedt, 1930, p. 688, fig. 390; Subrahmanyam, 1946, p. 134, figs. 214, 215.

Cells linked in chains; transapical axis shorter; middle of the valve possesses a knob (Fig. 19).

Length apical axis, 34.5  $\mu$ .

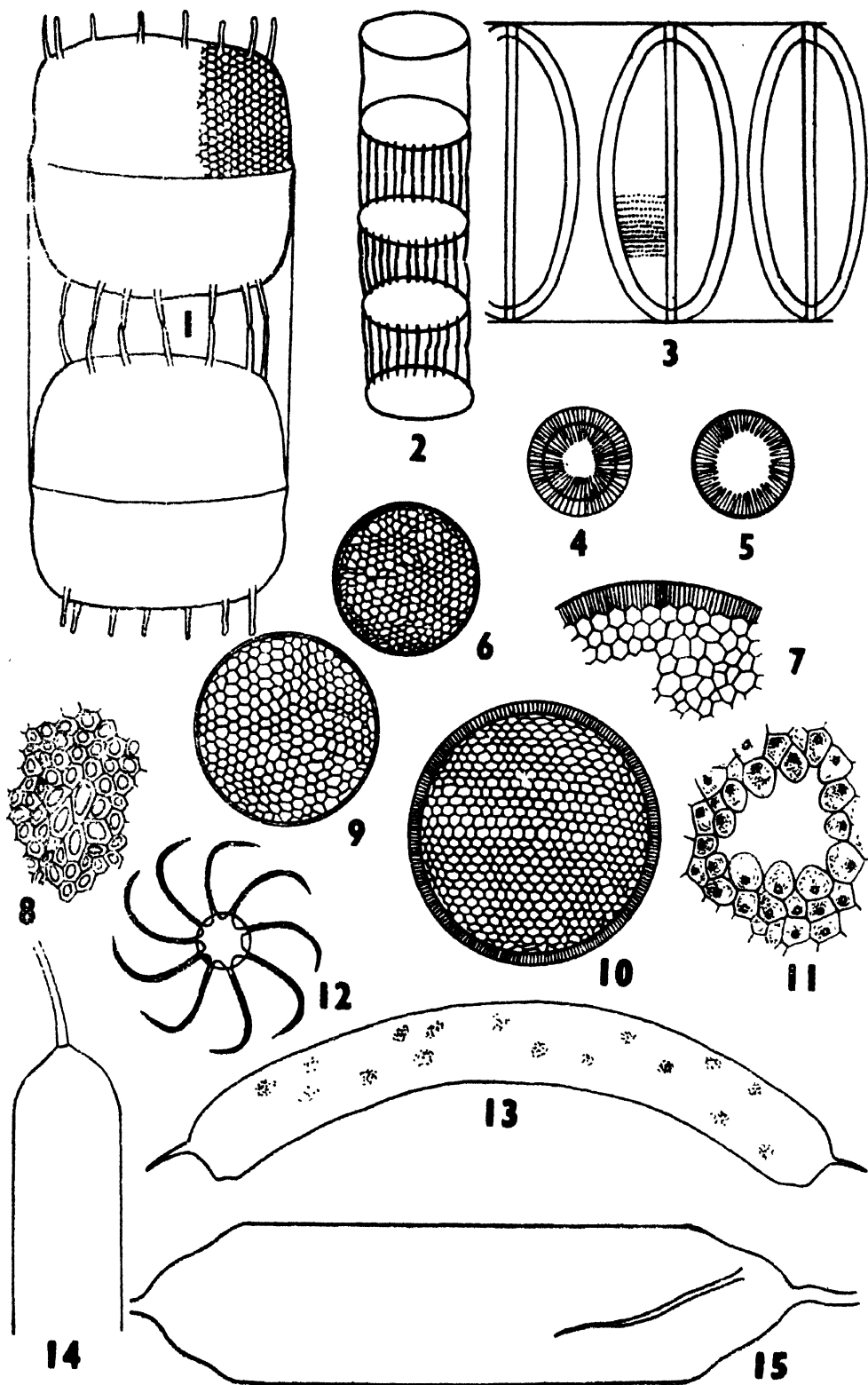
Habitat: Planktonic. June 15, 1956.



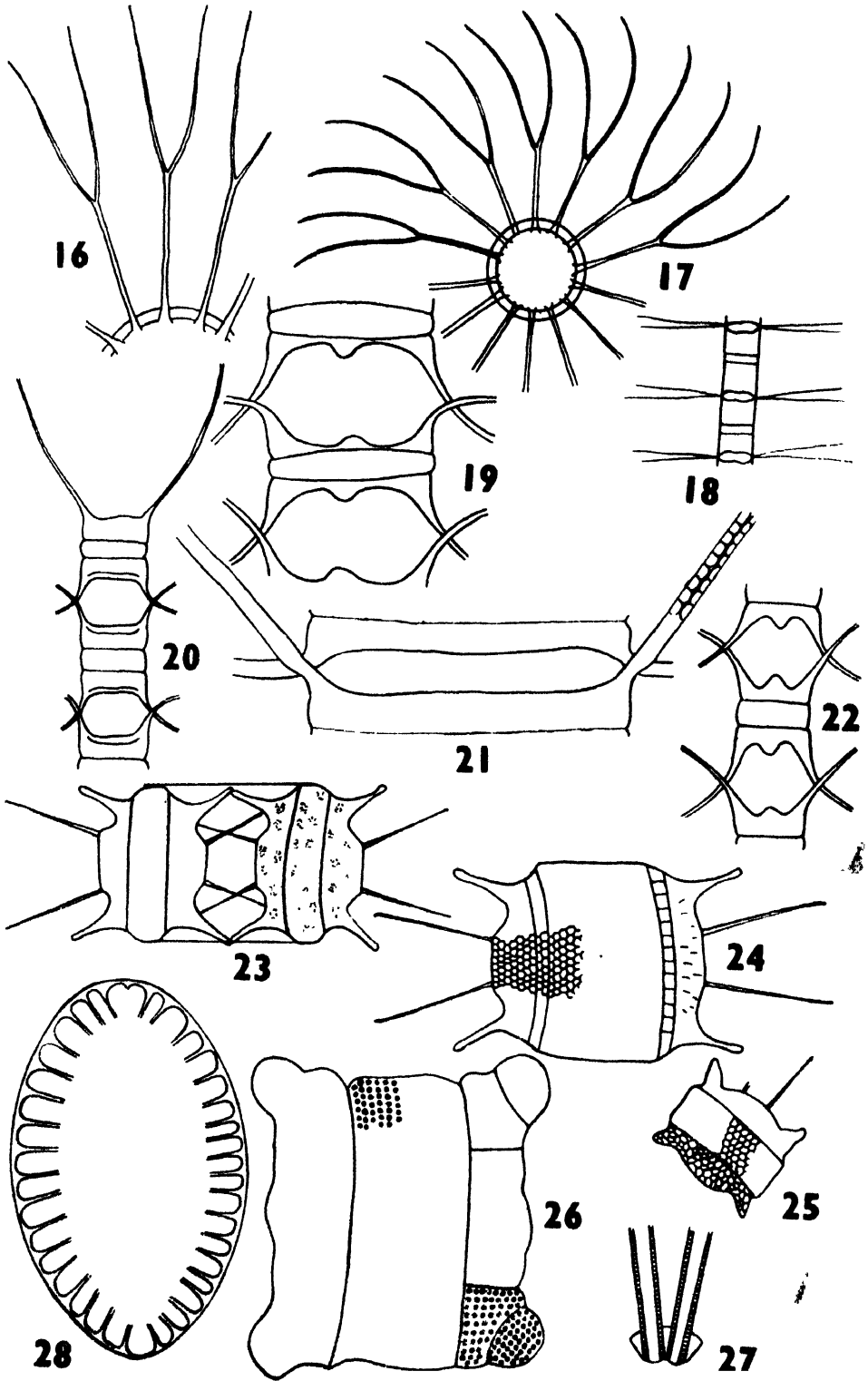
22. *Chaetoceros didymus* var. *protuberans* (Lauder) Gran et Grendo. Hustedt 1930, p. 690, fig. 392; Subrahmanyam, 1946, p. 135, fig. 216.  
Cells linked in chains; terminal setae thicker (Fig. 22).  
Length apical axis, 13.3–15.2  $\mu$ .  
Habitat: Planktonic. June 15, 1956.
23. *Biddulphia heteroceros* Grunov. Subrahmanyam, 1946, p. 155, fig. 188; 298, 303.  
Cells squarish or box-shaped; polar horns strong; valves between spines slightly higher; valve surface possesses many small spines; both valve and girdle areolated (Fig. 24).  
Length apical axis, 45–48  $\mu$ .  
Areolae. 9.10 in 10  $\mu$ .  
Habitat: On bottom mud of saline pools. June 18, 1956.
24. *Biddulphia mobiliensis* Bailey. Hustedt, 1930, p. 840, fig. 495; Subrahmanyam, 1946, p. 155, figs. 286–87, 291–6, 299; Pl. II. figs. 1–2.  
Cells linked in small chains by means of their horns; each valve possesses two long slender spines: valve flat between the spines; girdle and valve areolated (Fig. 23).  
Length apical axis, 26.6–76  $\mu$ .  
Habitat: On bottom mud of saline pools. June 18, 1956.
25. *Biddulphia pulchella* Gray. Hustedt, 1930, p. 832, fig. 490; Misra, 1956, p. 544, fig. 17.  
Valves elliptical, spinose and provided with long sutures; membrane areolated; ocell margin undulating; middle portion of the valve raised (Fig. 26).  
Length apical axis, 95–152  $\mu$ .  
Areolae, 5–8 in 10  $\mu$ .  
Habitat: Epiphytic on *Chaetomorpha* sp. June 17, 1956.
26. *Biddulphia rhombus* (Ehrén) W. Smith. Van Héurek, 1899, p. 472, Pl. XX. fig. 634; Hustedt, 1930, p. 842, figs. 496–7; Subrahmanyam, 1946, p. 157, figs. 285, 290, 302.  
Valves elliptic-lanceolate, valve silicious; areolated, horns stout; valves with two long spines; girdle view rectangular (Fig. 25).  
Length apical axis, 26.6–34.2  $\mu$ .  
Areolae on the valve, 9 in 10  $\mu$ .  
Areolae on the girdle, 12–14 in 10  $\mu$ .  
Habitat: From the scrappings of the pebbles immersed in sea-water. June 17, 1956.

## TEXT-FIG. 1

Figs. 1–15.—Fig. 1, *Stephanophyxis palmeriana* (Grov) Grunov. Fig. 2, *Skeletonema costatum* (Grev) Cleve. Fig. 3, *Hyalodiscus scoticus* (Kütz) Grun. Fig. 4, *Cyclotella striata* (Kütz) Grun. Fig. 5, *Cyclotella meneghiniana* Kütz. Fig. 6, *Thalassiosira decipiens* (Grun) Jörg. Fig. 7, *Coscinodiscus finicus* Misra. margin of the valve. Fig. 8, *C. granii* Cough. Central rosette. Fig. 9, *C. sub-lineatus* Grunov. Fig. 10, *C. lineatus* Ehrén. Fig. 11, *C. asteromphalus* Ehrén. Central area. Fig. 12, *Bacteriastrum cosmosum* Pavillard. end cell, valve view. Fig. 13, *Rhizosolenia stollerforthii* Peragallo. Fig. 14, *R. cylindrus* Cleve. Fig. 15, *R. crassispina* Schroeder. (Figs. 1, 4, 5, 12, 14,  $\times 820$ ; Figs. 8, 10, 13, 15,  $\times 1140$ ; Figs. 2, 3, 6, 7, 9, 11,  $\times 2200$ ).



TEXT-FIG. 1



TEXT-FIG. 2.

27. *Thalassiothrix frauenfeldii* Grunov. Van Hëurek, 1899, p. 322, Pl. XXX, fig. 839; Hustedt, 1931-1932, p. 247, fig. 727; Subrahmanyam, 1946, p. 169, figs. 349, 351, 354-7, 360.

Frustules linked in stellate chains; poles dissimilar (Fig. 27).

Diam. frustule, 5.7-7.6  $\mu$ .

Length frustule, 190-277.4  $\mu$ .

Striae, 10-12 in 10  $\mu$ .

Habitat: Planktonic. June 15, 1956.

28. *Surirella ovalis* Brébisson. Hustedt, 1930, Heft 10, p. 441, figs. 860, 861; Venkataraman, 1939, p. 357, fig. 139.

Valves ovate; costae short; striations radial; central space indistinct (Fig. 28).

Diam. frustule, 22.8  $\mu$ .

Length frustule, 38-76  $\mu$ .

Striae, 12-15 in 10  $\mu$ .

Habitat: Planktonic. June 15, 1956.

#### ACKNOWLEDGEMENTS

The author records his deep sense of gratitude to Dr. M. S. Randhawa for his keen interest and constant help in the preparation of this paper. He is grateful to Dr. B. P. Pal and Dr. S. M. Sikka for kindly providing all the facilities to carry out this work.

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#### TEXT-FIG. 2

Figs. 16-28.—Fig. 16. *Bacteriastrum hyalinum* Lauder. Fig. 17. *B. hyalinum* var. *princeps* (Cast) Ikari. Fig. 18. *Chaetoceros affinis* var. *intermedius* Subrahmanyam. Fig. 19. *C. didymus* Ehrén. Fig. 20. *C. laciniosus* Schütt. Fig. 21. *C. lorenzianus* Grunov. Fig. 22. *C. didymus* var. *protuberans* (Laud) Gran et Grendo. Fig. 23. *Biddulaphia mobiliensis* Bailey. Fig. 24. *B. heteroceros* Grunov. Fig. 25. *B. rhombus* (Ehrén) W. Smith. Fig. 26. *B. pulchella* Gray. Fig. 27. *Thalassiothrix frauenfeldii* Grunov. Fig. 28. *Surirella ovalis* Bréb. valve view. (Fig. 18,  $\times 420$ ; Figs. 16, 17, 23, 25, 27,  $\times 820$ ; Figs. 19-22, 24, 26,  $\times 1140$ ; Fig. 28,  $\times 2200$ ).

# NOTES ON THE HABITAT AND HABITS OF *CLEVELANDIA IOS* (JORDAN AND GILBERT)

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## ABSTRACT

A short description of the habitat of *Clevelandia ios* is given.

It is observed that while some of the larger individuals go into the burrows of *Urechis*, *Callinassus* or *Upogebia*, during low tide, the smaller ones remain in the pools formed. No accessory respiratory adaptations are noticed in the fish even though they retire into the burrows of the hosts, where the oxygen content of the water is lowered after the mud flats are exposed. Experiments have shown the *Clevelandia* is ourythermic.

Details of food and feeding habits are given. Analyses of stomach contents of specimens of different sizes collected over a period of twelve months show that there are changes in the diet with age and with season although copepods form the mainstay of food at all ages and at all seasons. During the periods when the copepods are low the fish seem to eat more of ova, nauplii, nematodes annelids, etc. The larger fish eat more of larger organisms and smaller items such as diatoms and tintinnids decrease with increasing size. There seems to be a gradual change from plankton to bottom feeding with advancing age.

## INTRODUCTION

*Clevelandia ios*, commonly known as the "arrow goby", belongs to the family Gobiidae. Actually a specimen of *C. ios* was first described as the type specimen of a new species (*Gobiosoma ios*) by Jordan and Gilbert in 1882. The genus *Clevelandia* was first erected by Eigenmann and Eigenmann (1888) and they described a new species, *C. longipinnis*. Jordan and Starks (1895) referred *ios* to *Clevelandia*. Jordan (1896) described a supposed new species, *Clevelandia rosae*, from San Diego. Jordan and Evermann (1898) recognised and described two species of the genus *Clevelandia*, *C. ios* and *C. rosae*. The specific name *longipinnis* was considered to be preoccupied, but since they believed the two species *longipinnis* and *rosae* to be identical, they used the latter specific name. Jordan, Evermann and Clark (1930) recognised only one species of this genus, *C. ios* and all the other names have been reduced to synonyms.

In distribution this species ranges from Lower California to the Strait of Georgia in Southern British Columbia. In California they have been recorded as occurring in the following localities : Bodega Lagoon, Tomales Bay, Butano Creek, Carquinez Strait in Solano County, San Pablo Bay, San Francisco Bay, Mouth of Salinas River, Elkhorn Slough, Morrow Bay, fresh tide-water region of San Gabriel River and Morrow Creek in San Luis Obispo County, in the *esteros* at Carpinteria and Goleta in Santa Barbara County, Mugu Lagoon in Ventura County, Mission Bay, San Diego Bay and Tijuana Slough in San Diego County.

## MATERIAL AND METHODS

The material for this study was collected from Elkhorn Slough, a tributary of Monterey Bay (36° 48' 45" N ; 121° 47' 15" W) from July 1946 to February 1948.

<sup>1</sup> Present address : Central Marine Fisheries Research Station, Mandapam Camp, South India.

In July and August 1946 a regular "minnow seine" was used for collection but from September onwards a small "hobinet" seine measuring 255 cm. in length and 94 cm. in depth was adopted. The meshes of this net are approximately round, measuring 2 mm. in diameter.

The fish were measured after they had been killed in a 5 per cent formaldehyde solution. A pair of needle-point dividers were set to the length to be determined and the reading made on ruler marked into half-millimeter units. The measurements were expressed to the nearest quarter of a millimeter by estimation.

#### HABITS AND HABITAT

*Clevelandia ios* is a small active fish that lives both free and commensally in the borrows of *Urechis*, *Upogebia* and *Callianassa*. They prefer a bottom composed of a mixture of sand and mud in shallow waters where the wave action is slight. As a typical habitat mention may be made of Elkhorn Slough, a tributary of Monterey Bay, where they are most abundantly found<sup>1</sup>, especially in the region of the clayey sand. The ecological conditions of the Slough have been described in detail by MacGinitie (1935). Since the publication of his work, the conditions of the Slough remained more or less the same, as described by MacGinitie, until September 1946 when an opening was made through the long sand spit in order to facilitate the fishing boats to enter the Slough. Fig. 1 shows the place where the opening was made (X). Further north a causeway was built across the Slough and at present the water on either side of the causeway communicates through a pipe 3 feet in diameter. The new opening to the sea and the partial shutting off of the old communication with the sea, by the construction of the causeway, have brought about changes in the nature of the Slough. Before the new opening was made, escape of water from the Slough during low tide was delayed or prevented by the formation of a sand bar at the mouth of the Slough. This resulted in a greater portion of the Slough being covered with water. The water that now comes in through the new opening during high tide flows out rapidly during low tide because of the deepening of the channel and the lack of formation of a bar. This leaves the major part of the Slough completely exposed except for a channel on the western side and a few shallow pools here and there.

The ground colour of *Clevelandia* in life is pale olivaceous to grey, spotted with black pigments. Closer examination shows that xanthophores are present associated with many of the melanophores. Irridescent white spots, varying in number, are found along the mid-lateral region and sometimes also on the opercula. The colour matches perfectly with the environment and it is difficult to locate the fish when it is at rest. The fish is also capable of changing its colour as are many other fishes (Mast, 1914). On a light background it turns pale whereas on a dark background it quickly changes to a darker shade.

*Clevelandia*, when disturbed, retreats into the burrows of *Urechis*, *Upogebia* or *Callianassa*. The burrow is used as a shelter rather than as a residence. When the tide goes out they do not generally migrate into deeper waters, but remain either in the pools or in the burrows of one of the hosts mentioned until the next high tide covers the area. On such occasions, when the beds of these hosts are exposed, the gobies can be collected by digging and as many as five gobies have been taken from a single burrow. Only the larger individuals go into the burrows. In this connection it is worth quoting the observations made by MacGinitie (1934) : "*Clevelandia ios*, a small goby fish, is a very interesting creature. It not only seeks shelter within the burrows of the above-mentioned hosts, but, as observed in the laboratory, it makes inspection trips throughout the length of the burrows,

<sup>1</sup> Dr. Bolin informs me (personal communication, January 1955) that they are not particularly common in this area any more,

being perfectly at home at any depth and wriggling past its host whenever it wishes, to the entire indifference of the latter."

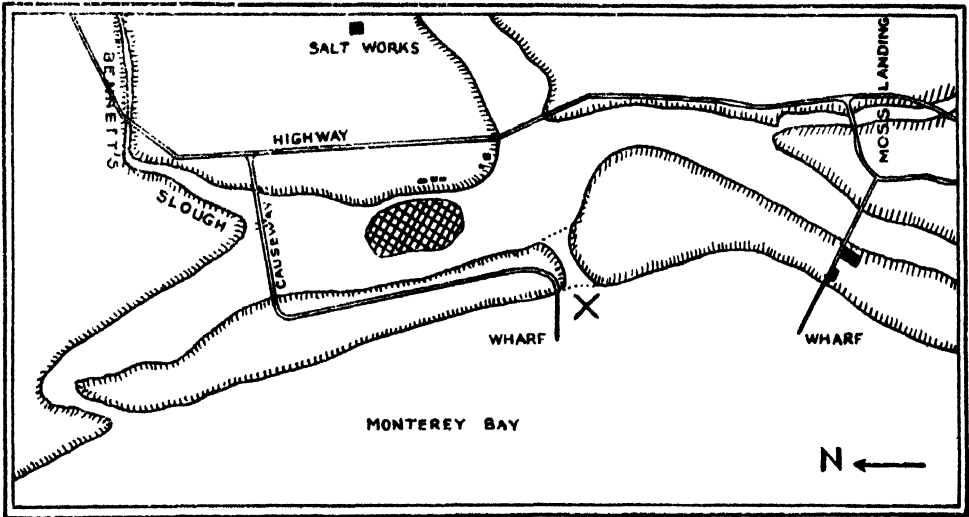


Fig. 1. Map of Elkhorn slough. The cross hatched region indicates the area where samples of *Clevelandia* were collected for this investigation. (After MacGinitie, 1935 with necessary modifications).

The movements of *Clevelandia* are not different from those of other species of gobies and it is believed that the fish uses its ventral fin to support itself on any surface. Duncker (1929) remarks: "Die Bewegungen der meisten *Gobius*-Arten sind auffällig ruckartig; von dem Punkt, an dem sie gegessen haben, schiessen die Tiere geradlinig zu einem anderen, um dort sofort wieder still zu sitzen. Nicht nur an Steinen, Pfählen und dergleichen, sondern auch auf ebenem Sandgrund werden dabei die Bauchflossen der Unterlage angedrückt. Es ist nicht ganz leicht, sich von der Funktion der letzteren ein klares Bild zu machen. Beim ersten Ange-drücktwerden flachen die *V* sich natürlich ab; um jedoch eine Haftwirkung auszuüben, müssen sie einen Hohlraum negativen Drucks umschliessen. Die einzige, schwierig wahrzunehmende Bewegung der *V* unmittelbar nach dem Andrücken besteht in einer Abduktion ihrer Stacheln, durch welche der bisweilen gefranste Hinterrand der Trichtermembran gestrafft wird. Ob sich dadurch, wie zu erwarten, die Trichtermembran von der Ventralfläche der Bauchflossen abhebt und auf diese Weise den erforderlichen Hohlraum bildet, konnte ich nicht mit Sicherheit feststellen, ist mir jedoch nach Versuchen am Präparat wahrscheinlich. Die Haftfähigkeit besteht für alle Neigungswinkel der als Unterlage dienenden Flächen."

MacGinitie (1935) remarks: "By means of its pectoral fins it is able to cling to the side of the glass in an aquarium." To find out how *Clevelandia* clings to the glass in an aquarium, three simple experiments were conducted. In the first experiment the lower half of the pectoral fins was cut off so that the fins could not touch the surface but the ventral fin was left undisturbed. The clipping off of the pectoral fins does not seem to hamper the movements of the fish. The fish could still cling to the surface as usual and while it is clinging to the glass side of the aquarium the pectoral fins were not touching the surface.

Aquarium observations have shown that the fish first presses the ventral fin against the glass wall or the bottom and then gradually raises its body which at the same time slightly elevates the ventral fin, too. This gives one the impression that the fish is trying to create a vacuum, as is usually believed. To test this the transverse membrane at the base of the ventral fin was cut off to prevent the

formation of a vacuum. The result, as was expected, was that the fish could not cling to the side wall of the aquarium. In another specimen the ventral fin was cut longitudinally into two halves which also prevented the formation of a vacuum. The same result was observed. From these it is concluded that *Clevelandia*, and probably all the other gobies with a united ventral fin, use the ventral fins and not the pectoral fins for clinging to a surface. This is brought about by the creation of a partial vacuum inside the sucker-like ventral fin.

Fighting amongst *Clevelandia* has been noticed only once. A very interesting combat was observed one day while feeding them. A few copepods were dropped into the aquarium. Two female gobies having simultaneously tried and failed to catch the same copepod, lay quiet for a second, then opened their mouths to the fullest extent and charged head on. Their jaws interlocked, one biting the upper jaw of the other and the other the lower jaw of its opponent. Holding this grip they raised about two-thirds of their body from the bottom. This was followed by violent shaking and wriggling movements of the body. Following this the combatants freed themselves, moved back and charged once again. The same procedure was repeated three times after which the fight stopped for a while and they moved about picking up the copepods. But when the same two individuals met again face to face, they picked up the fight once more. The final result was that one of them lost a part of its lower jaw.

The fight has nothing to do with parental care during which period gobies have been observed to attack other gobies or any intruder who might disturb the eggs.

The habit of *Clevelandia* retiring into the burrows of *Urechis* during low tide when the mud flats are exposed, brings up the interesting question of specialized respiratory organs. Redfield and Florkin (1931) have shown that the oxygen content of the water in the burrows decreases rapidly during the first hour after the flat is exposed and, by the fourth hour, to reach a minimal value of 0.06 c.c. per 100 c.c. of water. During the fifth hour there is a definite increase in the oxygen content of the water. Thus there is a possibility of integumental respiration being carried on by *Clevelandia* during this period of low oxygen concentration as found in such species of gobies as *Typhlogobius*, *Bathygobius* and *Gillichthys*.

In cases of well developed integumental respiration it is always observed that the blood vessels come as close to the surface of the integument as possible to facilitate easier and quicker diffusion of the gases. The diffusion of gases is, of course, inversely proportional to the thickness of the partition and hence the efficiency of diffusion is increased if the blood vessels come as close to the surface of the integument as possible. Many examples of integumental respiration have been given by Schoettle (1931). An examination, even with the naked eyes, of those species where there is definite integumental respiration will show a superficial network of blood vessels. *Typhlogobius californiensis* has a subepidermal network of blood vessels (Ritter, 1893). *Bathygobius soporator* has vascularized pectoral fins (Beebe, 1931), and the jaw membrane of *Gillichthys mirabilis* shows rich vascularization (Weisel, 1947). *Clevelandia ios* does not have any such rich subepidermal vascularization. This has been proved by the study of microtome sections of the operculum, pectoral fin, body wall and the tissue covering the head. It can be said with certainty that there is no specially developed integumental respiration in *C. ios*.

MacGinitie (1935) suggests: "They use their operculi but little, and it is probable that they respire mainly through the integument." On the other hand Wilby (1933) has observed: "The rate of breathing varies considerably." He studied the fish in aquaria. The fish buried themselves in the sand and soon made a water passage one on either side of the body, immediately behind the head, by a few slow expansions of the under side of the head followed by rapid contractions. He goes on to say: "After burial the first regular pulsations were about 40 per minute. As soon as a water passage had been made the rate increased to



about 50 or 60 varying with the different individuals. At a temperature of about 25°C the fish were still alive but apparently in distress and the breathing rate increased to about 80 per minute."

The observations the author made on respiration of *Clevelandia* were at a temperature of 13.5°C. The number of breathing movements of the opercula were counted for six individuals at rest. It varied from 36 to 62 per minute.

One would normally expect *Clevelandia* to be eurythermic, placed as they are in an environment which is subject to wide fluctuations in temperature. Experiments were conducted in the laboratory to ascertain the tolerance of *Clevelandia* to changes in temperature. A number of individuals collected from Elkhorn Slough, were subjected to different temperatures for a maximum period of sixty hours and the mortality rate observed. The range of temperature varied from 4°C to 33.5°C. The results of the experiment are given in Table 1.

TABLE 1

*Effect of temperature on Clevelandia ios.*

| Number of fish | Range of temperature | Time Hours | Observations at the end of the time indicated in the left hand column  |
|----------------|----------------------|------------|--|
| 13             | 4.0°C                | 4          | Slightly inactive, but when disturbed swam around actively. 2 of the 13 specimens lay upside down, but when disturbed they turned around and swam about. |
|                |                      | 16         | Inactive, 50 per cent of them lay with their belly up, but none of them was dead.  |
|                |                      | 22         | One dead and the others showed signs of slight distress and were sluggish.   |
|                |                      | 40         | All alive but very sluggish.   |
|                |                      | 60         | All alive but very sluggish.   |
| 4              | 17°-18°C             | 4          | All alive and active.  |
|                |                      | 16         | All alive and active.  |
|                |                      | 22         | All alive and active.  |
|                |                      | 40         | All alive and active.  |
|                |                      | 60         | All alive and active.  |
| 13             | 22°-26°C             | 4          | All alive and active.  |
|                |                      | 16         | All alive and active.  |
|                |                      | 22         | A few showed slight distress and swam about in circles.  |
|                |                      | 40         | All alive and seemed apparently all right.   |
|                |                      | 60         | All alive and active.  |
| 13             | 29°-33.5°C           | 4          | One dead and the others showed slight distress.  |
|                |                      | 16         | Ten dead and the others showed increasing distress and restlessness.   |
|                |                      | 22         | Two more out of the remaining 3 died.  |
|                |                      | 40         | All dead.  |

From these observations it is evident that the low temperature did not suit them well and the extreme sluggishness of the fish is evidence of this. They behaved normally and remained very active throughout in the 17° to 18°C range, whereas from 22° to 26°C even though all specimens survived to the end of the

experiment, the fish began to show signs of distress, but they soon got over it and regained their normal activity. This is perhaps due to the fact that they became acclimated to the higher temperature. Wilby (1933) has stated that the fish survived at a temperature of about 25°C but apparently were in distress. As the temperature increases (29° to 33.5°C) the ability of the fish either to withstand it or to get acclimated to decreases and results in death.

In Elkhorn Slough the usual range of temperature is from 12° to 19°C. But it has been noticed that in the pools that are left when the tide recedes the temperature of the water rises, in the sunshine, to 23°C. However, at the same place where the temperature of the water was 23°C, the temperature in the underlying mud, at a depth of six inches, was only 15°C. The highest temperature recorded in such mud was only 19°C. These figures are taken from MacGinitie (1935). Thus the bottom living *Clevelandia* has to encounter in nature only a range of temperature from 12° to 19°C. The experiments at the same time show that they can withstand a wider range of temperature even though they are most active in the 17° to 18°C range.

#### FEEDING HABITS AND FOOD

The feeding habits of adult *Clevelandia* have been observed in the aquarium. Different kinds of food, such as small Annelids, pieces of Phoronids, small shrimps, pieces of crab and mussel meat and copepods were readily taken. Algal filaments, which were provided, were seldom eaten. Even though they pick up any kind of material, the particles are not eaten always unless they are of the right type of food material. MacGinitie (1934) observed in the laboratory: "If a large piece of, for example, clam meat, too large for a goby to swallow, is put into the burrow which also contains crabs, the goby, after attempting to swallow or tear it apart, will carry the meat to a crab and stand by while the latter makes it smaller, the fish at intervals snatching it for another attempt at swallowing, to the disconcertion of the crab. The fish will do the same thing in an open aquarium with *Spirontocaris* present." It was observed that the fish prefer moving objects and generally pick up the animals from the bottom. Occasionally they come up and feed on the animals sticking to the sides of the aquarium but they have never been noticed to feed from the surface of the water. The food material is picked up from the bottom by a quick lateral movement combined with a slight forward jerk or a straight front attack using their pectoral fins for the forward movement. Feeding seems to be confined to day time as they have not been seen feeding at night.

The stomach contents of thirty specimens, including the smallest and the largest in the catch, were examined each month soon after the fish had been killed and preserved in a 5 per cent formaldehyde solution. The alimentary canal of each specimen was dissected out and the contents, spread out on a slide, were examined. No attempt was made at a thorough quantitative analysis and even in the qualitative analysis the usual procedure was to record only the genera of the organisms present. In a few cases the species also were determined when the condition of the organism permitted it. The organisms were recorded as 'present but few', 'present in fair numbers' and 'numerous'. Later relative numerical values (arbitrary) were assigned to these and graphed (Figs. 2 and 3).

**DIATOMS:** Out of the samples examined the percentage of individuals found feeding on diatoms varied from 0 to 57 per cent. From November to May they are fairly well represented in the food and the utmost utilization by the maximum number of individuals was observed in May. A larger proportion of diatoms form the food of the smaller fish. The following genera of diatoms were included in the food: *Coscinodiscus*, *Grammatophora*, *Gyrosigma*, *Melosira*, *Navicula*, *Nitzschia* and *Thalassiothrix*.

**FILAMENTOUS ALGAE :** Filaments of *Enteromorpha intestinalis* were occasionally seen as part of the food. Only fourteen out of 360 had them in the alimentary canal.

**TINTINNIDS :** The percentage of fish found feeding on Tintinnids varied from 3.3 to 47 per cent in the different monthly samples. The peak falls in August and September. They form only a minor item of the food but are present all the year round, at least in one specimen. They all belong to the genus *Favella*.

**OVA :** Small eggs ranging from 55 to 60 micra in diameter formed a fair proportion of the food of both the young ones as well as the adults. Except in September and January, eggs were present in varying proportions as part of the food in all months. An analysis of the samples shows that from 26.6 to 87.7 per cent of the individuals were feeding on these ova. Identification of these ova proved impossible. Lebour (1919) mentions ova as forming a large proportion of the food of young fish. The diameter of the eggs, she observed, was about 1.6 mm. and since the size approximates that of the eggs of *Calanus finmarchicus*, which has free eggs, she believes that the eggs are of this species of copepod.

**NEMATODES :** Nematodes belonging to the genus *Metoncholaimus*, resembling *M. pristurus*, form a large proportion of the diet all the year round. Mostly the larger specimens feed on them. In the samples examined the percentage of individuals feeding on Nematodes varied from 3.3 to 66.7 per cent. Large number of this free living Nematode are found in Elkhorn Slough and they are doubtless food organisms and not parasites.

**ANNELIDS :** Annelids, either larval or otherwise, do not form an important item of the food. Out of 360 specimens examined only nine contained Polychaetes. They belonged mostly to the species *Capitella capitata*, and a few post-larval Polychaetes were also found. It is interesting to note here that out of 360 specimens examined for the year, fourteen of them were found to feed on the little known annelid belonging to the genus *Dinophilus*.

**CRUSTACEANS :** The most important item of the food is the crustaceans. Copepods of different species were found to be the most predominant among these and such larval forms as nauplii and zoea, especially the zoea stage of *Callinassa*, were also found occasionally. Only four out of 360 specimens contained no copepods. Copepods formed the staple food of the young as well as the adults throughout the year. Other crustaceans such as amphipods, isopods and *Nebalia* sp. were also included in the diet. The chief copepods forming the food were in two genera of Harpacticoids : *Canthocamptus* and *Pseudobralyn*.

In a few instances the stomach contents were so far digested that identification was impossible. In three of such cases it is believed that the partly digested material was *Urechis* larvae. MacGinitie (1935) remarks that *C. ios* undoubtedly eats great numbers of the larvae of *Callinassa*, *Urechis*, annelid worms, etc.

The analysis of the food of *Clevelandia* on the basis of size (Fig. 2) reveals the following interesting facts. Copepods constitute the main item of food practically at all sizes. The other crustaceans such as amphipods, isopods, etc., become important only to the larger fish. So also the nauplii, zoeae and ova seem to become more important with age. The data given in Fig. 2 also suggest that the peak for the smaller nauplii occurs in the smaller fish. The complete absence of nauplii and zoeae in the 40-45 mm. group is surprising but it should be pointed out here that only nine specimens were available in this size range. It is difficult to explain why the ova become more prominent in the larger size group, but it is likely that these being not actively moving may require a considerable period of learning on the part of the fish before they are taken and hence become prominent in the larger size groups. Diatoms and tintinnids seem to be most important at a size range of 15-30 mm. and the decline in the importance of these in the larger fish is probably due to the size factor. The nematodes and annelids, which are comparatively larger organisms, become more prominent in the older groups and it is possible that

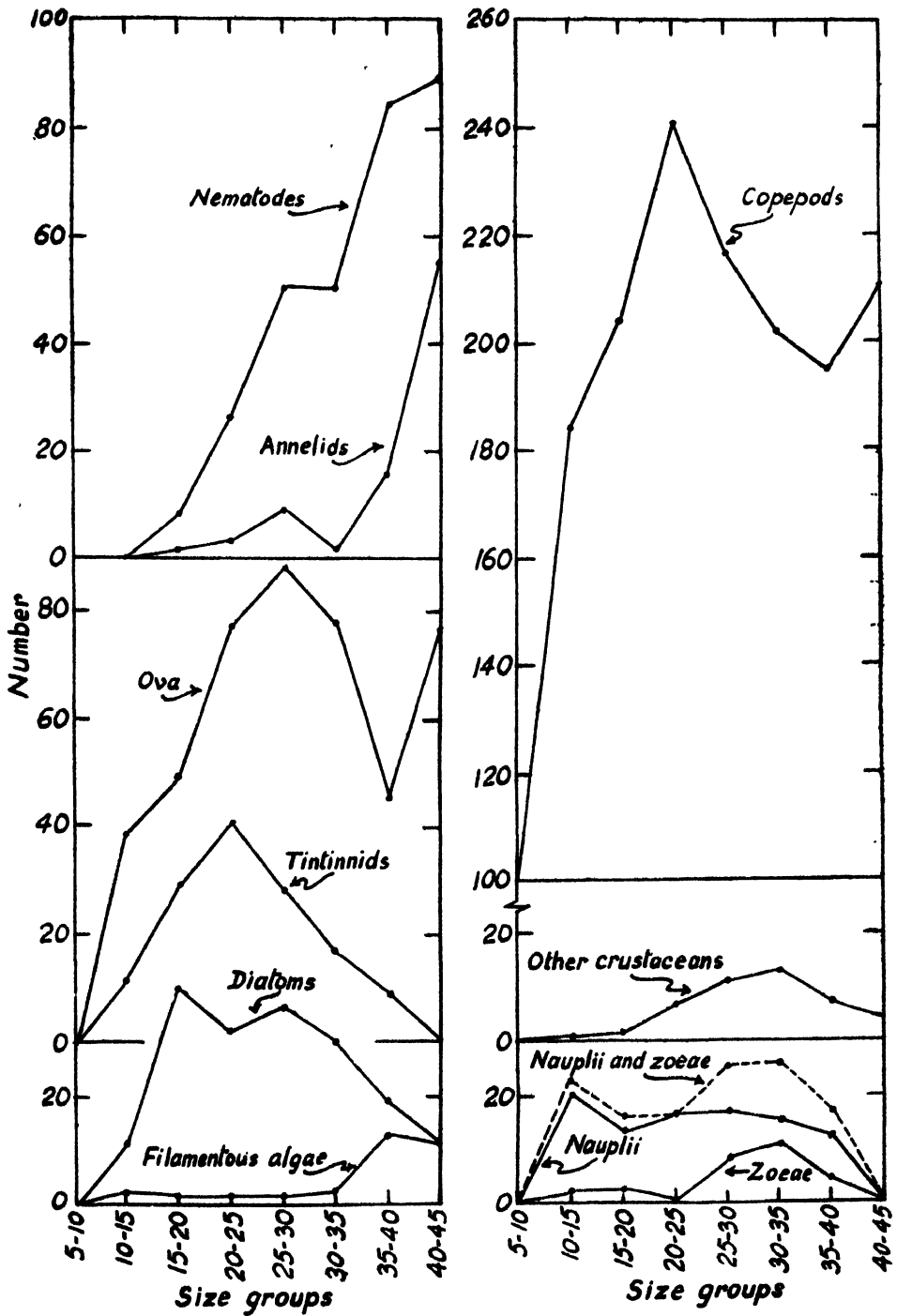
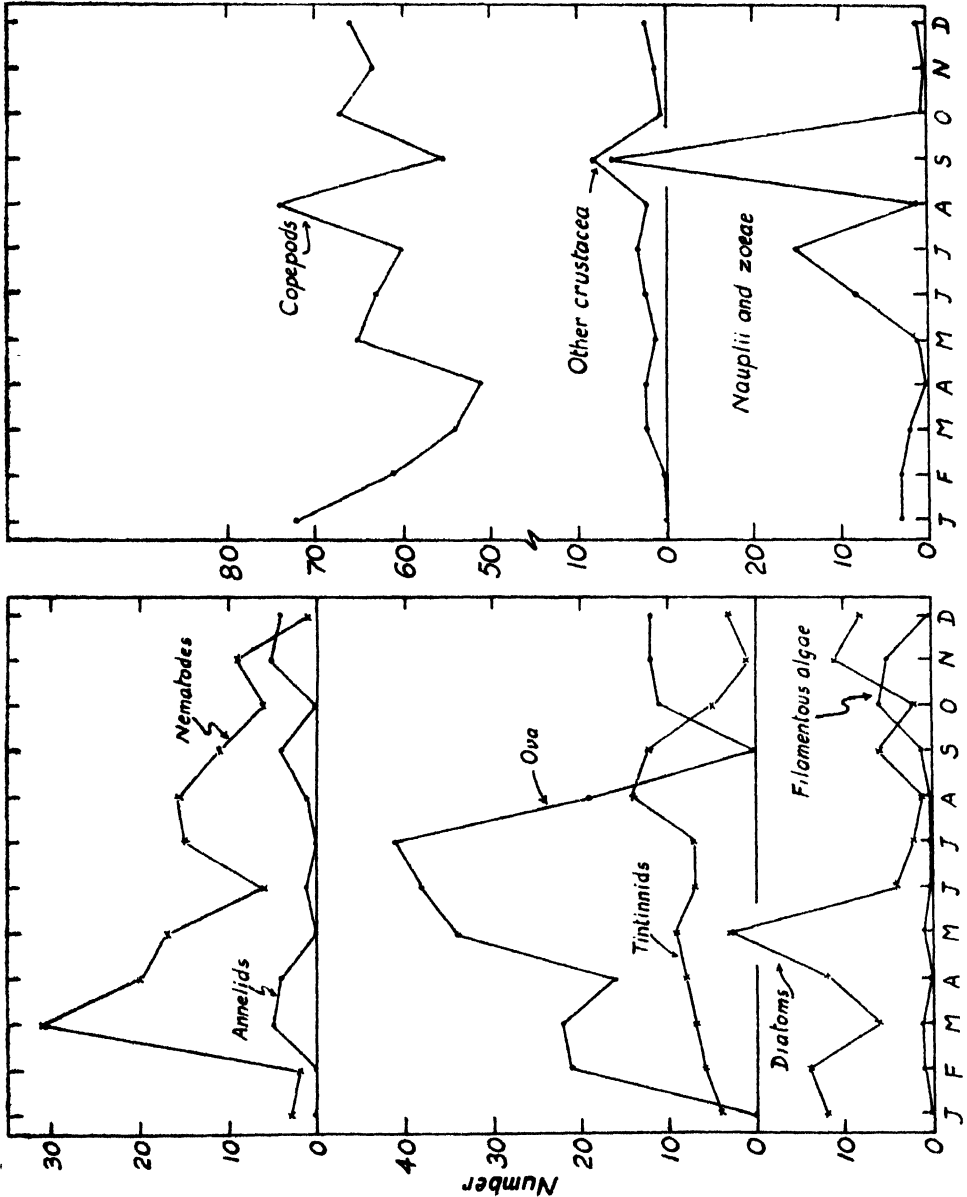


Fig. 2. Showing the food of *Clevelandia* at various size groups.

this may also mean a change from plankton to more of bottom feeding in the larger fish. Filamentous algae, which are negligible up to about 35 mm., show an increase in the larger fish. The presence of these may be incidental to catching the nematodes and annelids and seem to support the inference that the larger fish tend to feed more and more at the bottom. The observations in the laboratory also indicate that the adult fish feed more at the bottom.

Fig. 3 shows the seasonal variation in the food. Here again the copepods are the mainstay at all times and the other crustaceans show a small peak in September.



ber when the copepods show a decrease. From February to August there is a prominent peak of ova. It is likely that these are copepod eggs and the peak

probably is a reflection of the spawning of copepods. The nauplii and zoeae are found in fair numbers in June-July and in large numbers in September. If they are considered separately the peak of nauplii shows a tendency to occur in July and September which follows the peak of copepod eggs. The zoea peak on the other hand, occurs in September. These zoeae are, as mentioned earlier, of *Callinassa* and the peak coincides with their spawning. The nematodes show a prominent peak in March and are relatively abundant from March to September (with the exception of June when the value was low but this may be due to sampling error) and low values are obtained for the other months. It appears from this that the nematodes are more common during the warmer months when the fish feed on them actively. The annelid curve shows weak peaks in March-April and again towards the end of the year. The tintinnids, which form a minor item of food, show a distinct peak and it is interesting to note that the tintinnid curve is somewhat opposite to the diatom curve. This possibly suggests that when the diatoms are low the tintinnids are exploited as a substitute food. The diatoms show a distinct peak in summer and are fairly high during November-February and low during June to October which coincides fairly well with the period when the low nutrient oceanic water dominates the area. The filamentous algae, which are probably incidental to feeding at the bottom, show a very faint peak in October-November.

In summing up, it may be said that there are changes in the diet of *Clevelandia* with age and with season. The larger fish eat more of larger organisms and such smaller items as diatoms and tintinnids decrease with age. The increase in the number of nematodes and annelids, supported by the presence of more filamentous algae in the larger fish, suggests a gradual change from plankton to bottom feeding. The copepods form the mainstay of food at all times and at all seasons, but it was observed that during periods when the copepods are low, the fish eat more of nematodes, annelids, ova, nauplii, zoeae and other crustaceans like amphipods, isopods and *Nebalia*.

#### ACKNOWLEDGEMENTS

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ACHARYA JAGADIS CHUNDER BOSE (1858-1937)

## ACHARYA JAGADIS CHUNDER BOSE—HIS LIFE AND WORK (1858—1937)

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Acharya Jagadis Chunder Bose occupies a high, almost unique, place in the recent history of Indian science. He was an investigator of uncommon courage, resourcefulness and dedication. Bose's scientific work broadly falls under three periods. From 1894 to 1899 he was almost entirely concerned with the study of electric waves, between 1899 and 1902 he shifted from the physical to the bio-physical field, and beyond 1903 he was occupied with the study of plant-responses under physical stimuli of various types. For these studies he developed and constructed his own instruments which were remarkable for their originality and extreme sensitivity. Bose founded the *Bose Institute* in Calcutta in 1917. He continued to be the Director of the Institute till his death in 1937. Bose visited Europe many times, and America twice, on lecture tours. He was elected a Fellow of the Royal Society in 1920, and Corresponding Member of the Academy of Sciences, Vienna, in 1929. He was the General President of the Indian Science Congress in 1927. He served on the Council for Intellectual Co-operation of League of Nations from 1926 to 1930.

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Bose was born on November 30, 1858, in the town of Mymensingh in East Bengal. (His father, Bhagwan Chunder Bose, was at the time Deputy Magistrate of the place.) He died on November 23, 1937, at the age of 79 years. (He was survived by his wife, Mrs. Abala Bose. She was the daughter of Mr. Durga Mohan Das, a leading advocate of the Calcutta High Court.) He received his primary education at the local school at Faridpur: his father did not send him to the English school which was there in the same town. Later, he joined the St. Xavier's School in Calcutta, and the College, from which he graduated at the age of 20. He subsequently went to England and joined the London University to study medicine. He attended some lectures by the famous zoologist, Ray Lancaster. Due partly to reasons of health, he left London to join the Christ College at Cambridge. He studied for the Natural Science Tripos, and attended lectures, amongst others, by Lord Rayleigh (Physics). He took the Tripos (Cambridge) and B.Sc. (London) in 1884. On his return from England he was appointed Professor of Physics in the Presidency College, Calcutta, in spite of serious opposition from the then Education Department. Bose had to do as much as 26 hours of lecture and demonstration work per week. (This was much more than what was normal for his British colleagues in the same college.) He retired from the college in 1915.

It was probably at the age of about 35 that Bose seriously made up his mind to dedicate himself completely to the pursuit of science and scientific research. No grant at the time was available to him for research work. The laboratory in the Presidency College, Calcutta, was poorly equipped and sometimes Bose had to construct his apparatus from his own personal resources. It was several years later that the Government sanctioned for his work in the college an yearly grant of Rs. 2,500/-. Bose's earliest research work was concerned with electric waves and their interaction with matter. Electric waves were first produced in the laboratory by Heinrich Hertz in 1888 in his epochal experiments. The existence of these waves had been predicted by Maxwell about 20 years earlier on the basis of his extremely far-reaching and extraordinarily fruitful (as later work showed) electro-magnetic theory. It has been sometimes said that Bose was led

to the study of electric waves after reading a paper by Sir Oliver Lodge on "Heinrich Hertz and his Successors" (1894). From the very beginning Bose's remarkable physical insight, and his superb ingenuity and resourcefulness in experimentation were apparent. He succeeded in generating waves of wavelengths much smaller than what Hertz and others had done. He produced waves of about half-a-centimetre in wavelength. Because of this he was able to investigate in considerable detail the 'optical' properties of electric waves, such as refraction, polarisation and double refraction. He determined the refractive indices of many substances, and also investigated the influence on total reflection of the thickness of the air-gap between two dielectric slabs. In the paper published in the Proceedings of the Royal Society in November 1897 he observed: "It is seen from the above, that as the thickness of the air-space was gradually increased, the reflected component increased, while the transmitted portion decreased. Minimum thickness for total reflection was found to be 8 mm." He also verified that the thickness of the air-gap, for which total reflection disappeared, increased with the wavelength. It may be mentioned that Bose's first paper entitled "On Polarisation of Electric Waves by Double Refracting Crystals" (he tried beryl, rocksalt, etc.) was published in May 1895 in the Journal of the Asiatic Society of Bengal. In 1897 Bose gave a lecture at the famed Royal Institution, London. It is interesting (and also instructive) to recall that the demonstration apparatus exhibited at the lecture, which in present-day terminology may be described as a (simple) microwave spectrometer complete with transmitter and receiver (improved type of coherer), was constructed in Calcutta and taken by Bose with him to London. The originality and simplicity of the apparatus employed by Bose in his experiments were most remarkable. For instance, he demonstrated the polarisation of electric waves by the simple device of 'interleaving the pages of a Bradshaw railway time table with sheets of tin foil'. Again, to eliminate the undesirable reflections of electric waves in tubes employed to guide them (as in the case of spectrometer), he tried many different coatings—in other words, he was searching for an absorber of microwaves: he found that blotting paper dipped in electrolyte gave the best results. "Bose, in India between 1895-97, used hollow tubes of either circular or square section as waveguides and waveguide radiators on wavelengths between 5 mm. and 2.5 cm. His adoption of hollow tubes was probably based on the use of metal tubes in telescopes and microscopes".\* Bose also employed conical horns—he called them collecting funnels—for concentrating the waves on the detectors. He studied the rotation of the plane of polarisation, and found that a bundle of twisted jute fibres gave right or left-handed rotation depending on the right or left-handed twist of the fibres. This constituted a 'large-scale or macro demonstration' of the optical phenomenon of the rotation of the plane of polarisation.

For the detector, Bose used the coherer discovered by Branly and Lodge. He made considerable improvements, particularly in sensitivity and reliability. He also experimented with the point-contact-type detector consisting of a metal wire in contact with a metal plate or semiconducting crystal. In the case of most substances, the resistance falls when the detector is exposed to electric waves but there is also a rise of resistance for some substances such as lead peroxide and potassium. Bose found that in the case of galena crystal the detector was not only sensitive to electric waves but also to light radiation extending from infra-red to violet. Here he was obviously dealing with what later came to be recognised as photovoltaic effect. These experiments dealing with the variations in contact resistances under the influence of electric waves—particularly the erratic behaviour of the system in many cases—brought to Bose's mind the phenomenon of electric response in animal muscles when subjected to stimuli. "Bose enquires

\*J. F. Ramsay, 'Microwave Antennae and Waveguide Techniques before 1900', *Proc. I.R.E.*, Feb. 1958.

whether inorganic models may not also be devised which will satisfy this criterion. In this way he was able to construct models in which mechanical and light stimuli produce electrical responses. The proportionality which exists between intensity of stimulation and electrical response, the gradual appearance of fatigue in response after repeated stimulation, from which the system recovers after it is given sufficient rest, the increase of response on treatment with one set of chemicals and its inhibition by another set, are similar to what occurs in living tissues. We shall describe here only one of his models : it is made of two wires of pure tin, whose lower ends are clamped to an ebonite block; the upper ends pass through an ebonite disc, and are joined through binding screws to the two terminals of a sensitive galvanometer. The arrangement fits into a cylindrical glass vessel, filled with distilled or tap water. On giving one of the tin wires a sharp twist, an electric current flows from the wire through the galvanometer system. The amplitude of response is enhanced when a small quantity of sodium bicarbonate is added to the distilled water; on the other hand, if oxalic acid is added to the water the response is abolished. Many of the effects observed in animal tissues under stimulation, viz., of the opposite effects of small and large doses of a chemical poison, etc. could be obtained with this model of Bose.\* Mention here may also be made to the interesting analogy between the excitation of nerve and the passivity of iron dipped in strong nitric acid. This was investigated in great detail by Lillie (1920-36) and later by Bonhoeffer.† The first suggestion came from W. Ostwald in 1901. Another interesting model is due to Bredig (1903-1908) in which the oscillations of a mercury drop placed in a hydrogen peroxide solution appear (outwardly) to resemble the rhythmic pulsations of an animal heart.

These investigations gradually led Bose to the formulation of his fundamental concept (and in this context it is relevant to call attention to his early training in physiology and medicine) that basically the response, under stimulus, in the non-living (e.g., metal) and the living (e.g., animal muscle) is of the same nature, though they differ in their level of complexity. From about 1903 onwards Bose investigated with great ingenuity, vigour and perseverance the response phenomena in plants when exposed to various kinds of stimuli, e.g. mechanical, electrical and chemical, and also light radiation. He regarded that the response phenomena in plants lie between those exhibited in inorganic matter and in animals. He developed and constructed in his own laboratory special instruments for the purpose of measuring almost every type of plant-response. The rate of growth of plants is, crudely speaking, of the order of 0.1-0.01 mm. per minute, and to measure that he constructed many instruments which he named *Crescographs* (crescere : to grow). The high-magnification crescograph consisted of a combination of levers (in some cases mechanical and optical) giving a magnification of about 10,000. The magnetic crescograph, in which the small displacement of a magnet caused a large deflection in astatic magnetic system, produced a magnification of more than a million. Bose also developed several types of automatic recorders in which friction between the recording pen and the writing plate was eliminated by either vibrating the plate or the stylus. He constructed an instrument to record the liberation of oxygen during photosynthesis in plants. He also studied the variations, as a result of stimulations, in the electrical resistance of plant tissue. He was the first to use electric probes for the localization of actively metabolizing layers in plants.

Bose's plant work was largely carried out with the *Mimosa* plant and with *Desmodium gyrans* (telegraph plant, the Indian name is *Bon Chural*). He studied even such things as the effect of load (placed on the leaf) on response to

\*Jagadish Chandra Bose : Birth Centenary Series III; D. M. Bose, *Science and Culture*, 24, 5 (1958), p. 215.

†K. F. Bonhoeffer, 'On the Passivity of Iron' *Corrosion*, II (1955), See also R. Fatt, 'Physics of Nerve Processes'; Reports on Progress in Physics XXI, (1958), p. 112..

stimulus. For instance, he observes : "The effect of load on the response of *Mimosa* is similar to that on the contractile response of muscle. With increasing load the height of response undergoes a progressive diminution with shortening of period of recovery. Within limits, the amount of work performed by a muscle increases with load. The same is true of the work performed by the pulvinus of *Mimosa*." In the case of *Desmodium gyrans* he observed that the detached leaflet continued to show rhythmic pulsations, the period being of the order of 2 minutes. The pulsation occurs between the temperature of about 17 deg. C and 45 deg. C. The pulsation is affected by chemical reagents and electric stimuli. Bose also investigated the problem of the ascent of sap in plants. He thought, contrary to the generally accepted view then and now, that this is brought about by peristaltic activity of the inner cortical cells in the plant stem, somewhat analogous to the activity of the animal heart.

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It may be observed that one of the most far-reaching concepts which has emerged from the biological and physiological researches during the present century is that all vital processes in living organisms can be (completely) understood in terms of physical and chemical laws governing material phenomena. (It appears—some think it is certain—that this is not likely to be the case in the realm of phenomena concerning the mind). Towards this realisation Bose made a pioneering and very important contribution. In one of the papers read (but not published) before The Royal Society in 1904 he observed : "From the point of view of its movements a plant may be regarded in either of two ways : in the first place, as a mysterious entity, with regard to whose working no law can be definitely predicted, or in the second place, simply as a machine, transforming the energy supplied to it, in ways more or less capable of mechanical explanation. Its movements are apparently so diverse that the former of these hypotheses might well seem to be the only alternative. Light, for example, induces sometimes positive curvature, sometimes negative. Gravitation, again, induces one movement in the root, and the opposite in the shoot. From these and other reactions it would appear as if the organism had been endowed with various specific sensibilities for its own advantage, and that a consistent mechanical explanation of its movements was therefore out of the question. In spite of this, however, I have attempted to show that the plant may nevertheless be regarded as a machine, and that its movements in response to external stimuli, though apparently so various, are ultimately reducible to fundamental unity of reaction."\* And further, to quote from his book 'Plant Response as a Means of Physiological Investigation' (1906) : "The phenomenon of life, then, introduces no mystical power, such as would in any way thwart, or place in abeyance, the action of forces already operative. In the machinery of the living, as in that of the non-living, we merely see their transformation, in obedience to the same principle of conservation of energy as obtains elsewhere; and it may be expected that, in proportion as our power of investigation grows, the origin of each variation of the living organism will be found more and more traceable to the direct or indirect action upon it of external forces, the element of chance being thus progressively eliminated, as the definite sequence of cause and effect comes to be perceived with an increasing clearness; and only, I venture to think, as this is worked out, can we learn to apprehend fully the true significance of the great Theory of Evolution."

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In his papers and books Bose gives very few references to previous and contemporary workers. This is partly, no doubt, due to the fact that he was in most

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\* 'Plant Response as a Means of Physiological Investigation' by Sir Jagadish Chunder Bose (1906), p. VIII.

cases exploring new ground. It should also be mentioned that "the priority of many of Bose's observations, *e.g.* positive and multiple responses, alike electrical and mechanical, and transmission of death excitation, is seldom given the acknowledgement due in current literature on plant physiology.....He has left behind nineteen volumes which form a record of the work carried out and directed by him over a period of nearly thirty-seven years." Bose was truly a great man of science and his pioneering spirit and work have played a vital rôle in the revival of scientific research in our country. But for all this he was more in the nature of a lone worker—a towering but isolated peak—rather than a builder himself of a school of scientific research. To conclude, we may quote his memorable words spoken at the end of the lecture at the Royal Institution (London) in January 1897 : "The land from which I come did at one time strive to extend human knowledge, but that was many centuries ago. It is now the privilege of the West to lead in this work. I would fain hope, and I am sure I am echoing your sentiments, that a time may come when the East, too, will take her part in this glorious undertaking; and that at no distant time it shall neither be the West nor the East, but both the East and the West, that will work together, each taking her share in extending the boundaries of knowledge, and bringing out the manifold blessings that follow in its train."

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Geddes in his 'Life of Bose' gives the following extract from the *Spectator* (London) : "We can see no reason, whatever, why the Asiatic mind, turning from its absorption in insoluble problems, should not betake itself ardently, thirstily, hungrily, to the research into Nature which can never end, yet is always yielding results, often evil as well as good, upon which yet deeper inquiries can be based. If that happened—and Professor Bose is at all events a living evidence that it can happen—that would be the greatest addition ever made to the sum of the mental force of mankind."









